

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
11 December 2003 (11.12.2003)

PCT

(10) International Publication Number  
**WO 03/102131 A2**

(51) International Patent Classification<sup>7</sup>:

C12N

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(21) International Application Number: PCT/US03/12626

(22) International Filing Date: 22 April 2003 (22.04.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/374,722	23 April 2002 (23.04.2002)	US
10/157,580	29 May 2002 (29.05.2002)	US
60/386,782	6 June 2002 (06.06.2002)	US
60/398,036	23 July 2002 (23.07.2002)	US
10/225,023	21 August 2002 (21.08.2002)	US
60/406,784	29 August 2002 (29.08.2002)	US
60/408,378	5 September 2002 (05.09.2002)	US
60/409,293	9 September 2002 (09.09.2002)	US
60/440,129	15 January 2003 (15.01.2003)	US
PCT/US03/05190	20 February 2003 (20.02.2003)	US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NUCLEIC ACID MEDIATED DISRUPTION OF HIV FUSOGENIC PEPTIDE INTERACTIONS

(57) Abstract: The present invention relates to nucleic acid aptamers that bind to HIV envelope glycoprotein, gp120 and/or gp41 and methods for their use alone or in combination with other therapies, such as HIV RT inhibitors and HIV protease inhibitors. Also disclosed are nucleic acids such as siRNA, antisense, and enzymatic nucleic acid molecules that can modulate the expression of HIV env genes and HIV viral replication. The compounds and methods of the invention are expected to inhibit HIV viral fusion, cell entry, gene expression, and replication.

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## NUCLEIC ACID MEDIATED DISRUPTION OF HIV FUSOGENIC PEPTIDE INTERACTIONS

This application is a continuation-in-part of PCT/US03/05190 filed February 20, 5 2003, which claims the benefit of McSwiggen *et al.*, U.S. Provisional Application Serial No. 60/398,036, filed July 23, 2002, of McSwiggen U.S. Provisional Application Serial No. 60/374,722, filed April 22, 2002, of Beigelman U.S. Provisional Application Serial No. 60/358,580, filed February 20, 2002, of Beigelman U.S. Provisional Application Serial No. 60/363,124, filed March 11, 2002, of Beigelman U.S. Provisional Application 10 Serial No. 60/386,782, filed June 6, 2002, of Beigelman U.S. Provisional Application Serial No. 60/406,784, filed August 29, 2002, of Beigelman U.S. Provisional Application Serial No. 60/408,378, filed September 5, 2002, of Beigelman U.S. Provisional Application 15 Application Serial No. 60/409,293, filed September 9, 2002, and of Beigelman U.S. Provisional Application Serial No. 60/440,129, filed January 15, 2003 and which is a continuation-in-part of McSwiggen *et al.*, U.S. Patent Application Serial No. 10/225,023, filed August 21, 2002, which is a continuation-in-part of McSwiggen *et al.*, U.S. Patent Application Serial No. 10/157,580, filed May 29, 2002, which claims the benefit of McSwiggen U.S. Provisional Application Serial No. 60/294,140, filed May 29, 2001. These applications are hereby incorporated by reference herein in their entireties, 20 including the drawings.

### BACKGROUND OF THE INVENTION

The present invention concerns compounds, compositions, and methods for the study, diagnosis, and treatment of degenerative and disease states related to Human Immunodeficiency Virus (HIV) infection and/or acquired immunodeficiency syndrome 25 (AIDS). Specifically, the invention relates to nucleic acid molecules used to inhibit HIV cell fusion and entry via disruption of fusogenic peptide interactions.

Human immunodeficiency virus type I (HIV-1) enters permissive cells by binding to the cellular receptor, CD4, followed by fusion of the viral and target cell membranes. Fusion results in viral entry into the target cell followed by integration and expression of 30 the HIV-1 genome. The HIV-1 envelope glycoprotein mediates the fusion process

through interaction with cellular receptors. The HIV-1 envelope glycoprotein is synthesized as a precursor protein, gp160, which is proteolytically processed to generate two subunits, the surface glycoprotein gp120 and the transmembrane glycoprotein gp41. These subunits remain noncovalently associated to form the oligomeric envelope 5 glycoprotein spike on the viral membrane. Portions of gp120 bind to the CD4 receptor and a chemokine receptor (typically CCR5 or CXCR4) on the surface of target cells. These events trigger gp41 to undergo conformational changes that promote fusion of viral and cellular membranes, resulting in entry of the viral core into the cell. By analogy with the pH-induced structural changes in the hemagglutinin (HA) protein of influenza virus, 10 the HIV-1 fusion activation process likely involves substantial conformational changes from a pre-fusogenic state to a fusogenic conformation.

The structure of the ectodomain of both HIV and SIV gp41 in the fusogenic/post-fusogenic state has been characterized by NMR and crystallography. These studies have shown that gp41 consists of a trimer of hairpins. In the fusogenic conformation of gp41, 15 three N-terminal helices form a trimeric coiled coil, and three C-terminal helices pack in the reverse direction into three hydrophobic grooves on the surface of the coiled coil, bringing the amino and carboxy termini of the ectodomain together. Because the membrane anchor and the fusion peptide of the gp41 ectodomain are embedded in the viral and target cell membranes respectively, the formation of a fusogenic hairpin 20 structure results in the colocalization of the two membranes. Peptides corresponding to the C-terminal region, referred to as C peptides, can specifically inhibit viral entry into target cells at nanomolar concentrations. One such peptide (T-20) is in clinical study and has shown antiviral activity in humans. T-20 binds to gp41 only after interaction of the envelope glycoprotein complex with the cellular receptors.

25 Jeffs *et al.*, International PCT Publication No. WO 01/51673, describes isolated portions of gp41 protein (DP107 and DP178 domains) that are used to inhibit interaction between gp41 and gp120 and prevent infectivity of HIV.

Soukchareun *et al.*, 1998, *Bioconjugate Chemistry*, 9, 466-475, describes the use of N-Fmoc-cysteine(S-thiobutyl) derivatized oligodeoxynucleotides for the preparation of 30 certain gp41 peptide hybrid oligonucleotides having membranotropic activities.

**SUMMARY OF THE INVENTION**

The present invention relates to nucleic acid molecules used to inhibit HIV cell fusion and entry via disruption of fusogenic peptide interactions. The invention also relates to nucleic acid molecules directed to disrupt the function of the HIV-1 envelope glycoprotein, such as to inhibit CD4 receptor mediated fusion of HIV-1. In particular, the present invention describes the selection and function of nucleic acid molecules, such as aptamers, capable of specifically binding to the HIV-1 envelope glycoprotein and modulating activity of the HIV-1 envelope glycoprotein or components thereof. These nucleic acid molecules can be used to treat diseases and disorders associated with HIV infection, or as a prophylactic measure to prevent HIV-1 infection.

The nucleic acid aptamers of the invention can be used as antifusogenic and antiviral agents. The antifusogenic activity of the aptamers of the invention can result from the ability to modulate intracellular processes that involve coiled-coil peptide structures or protein-protein interactions. The antiviral activity of the aptamers of the invention includes but is not limited to the inhibition of HIV transmission to uninfected CD-4<sup>+</sup> cells.

The present invention also features the use of one or more nucleic acid-based techniques for modulating gene expression, such as nucleic acid aptamers, enzymatic nucleic acid molecules, small interfering RNA (siRNA), nucleic acid sensor molecules, allozymes, antisense nucleic acid molecules, 2,5-A nucleic acid chimeras, triplex oligonucleotides, and antisense nucleic acid molecules with nucleic acid cleaving groups, to modulate the activity, expression, or level of cellular proteins required for HIV cell fusion and entry. For example, the invention features the use of nucleic acid-based techniques to specifically modulate the activity and/or expression of proteins required for HIV cell fusion and entry.

In one embodiment, the invention features antifusogenic nucleic acid aptamers directed to disrupt the function of HIV-1 envelope glycoprotein or components thereof and prevent viral membrane fusion and/or entry. The nucleic acid aptamers of the invention are designed to interact with subunits of the HIV-1 envelope glycoprotein, such as the gp120 and gp41 subunits of the HIV-1 envelope glycoprotein, and disrupt the

function of the HIV-1 envelope glycoprotein or components thereof. Such disruption of the HIV-1 envelope glycoprotein can be effected, for example, by preventing conformational changes to gp120 or gp41, and/or preventing protein-protein interactions between gp120 and/or gp41 or interactions within gp120 and/or gp41.

5 In another embodiment, the invention features antifusogenic nucleic acid aptamers having binding affinity to gp41. Non-limiting examples of target regions within the gp41 peptide sequence include sequences derived from the C-terminal region of gp41. For example, the present invention features aptamers having binding affinity to a peptide sequence corresponding to amino acids 638 to 673 of GP-41, and aptamers having 10 binding affinity to a peptide sequence corresponding to amino acids 558 to 595 of GP-41 (see for example Jeffs *et al.*, US Patent Application No. 09/350,841, incorporated by reference herein in its entirety including the drawings).

In yet another embodiment, the invention features antifusogenic nucleic acid aptamers having binding affinity to peptide sequences having SEQ ID No. 1233 and/or 15 SEQ ID No. 1234 (**Table XIII**) or functional equivalents thereof. For example, in certain embodiments, nucleic acid aptamers of the invention can have binding affinity to analogs of the peptides contemplated herein, such analogs can contain one or more amino acid truncations, deletions, insertions or substitutions.

20 In one embodiment, the invention features an antifusogenic nucleic acid aptamer that specifically binds the HIV-1 envelope glycoprotein. In one embodiment, the invention features a nucleic acid aptamer that specifically binds the gp41 region of the HIV-1 envelope glycoprotein. In another embodiment, the invention features a nucleic acid aptamer molecule that specifically binds to the gp120 region of the HIV-1 envelope glycoprotein.

25 In one embodiment, nucleic acid aptamers of the invention act extracellularly and bind to their HIV-1 envelope glycoprotein targets outside of cells. These nucleic acid aptamers provide an attractive approach to treating HIV infection because they are able to act outside of cells or extracellularly.

30 In another embodiment, the invention features a composition comprising a nucleic acid aptamer of the invention in a pharmaceutically acceptable carrier. In another

embodiment, the invention features a mammalian cell, for example a human cell, comprising a nucleic acid aptamer contemplated by the invention.

In one embodiment, the invention features a method for treatment of HIV-1 infection and/or AIDS, comprising administering to a patient a nucleic acid aptamer of the invention under conditions suitable for the treatment.

In another embodiment, the invention features a method of treatment of a patient having a condition associated with HIV-1 infection, comprising contacting cells of said patient with a nucleic acid aptamer of the invention under conditions suitable for such treatment. In another embodiment, the invention features a method of treatment of a patient having a condition associated with HIV-1 infection, comprising contacting cells of said patient with a nucleic acid aptamer of the invention, and further comprising the use of one or more drug therapies under conditions suitable for said treatment. Examples of suitable drug therapies include reverse transcriptase inhibitors such as zidovudine (AZT), zalcitabine (DDC), zidovudine (ZDV), lamivudine (3TC), didanosine/delavirdine (DDI), stavudine (D4T), abacavir, efavirenz, nevirapine, or tenofovir disoproxil fumarate, ribavirin and/or protease inhibitors such as indinavir, amprenavir, saquinavir, lopinavir, ritonavir, or nelfinavir, or any combination thereof. In another embodiment, the other therapy is administered simultaneously with or separately from the nucleic acid molecule.

In another embodiment, the invention features a method for modulating HIV cell fusion in a mammalian cell comprising administering to the cell a nucleic acid molecule of the invention under conditions suitable for the modulation.

In yet another embodiment, the invention features a method of modulating HIV cell fusion, comprising contacting a nucleic acid aptamer of the invention with HIV-1 envelope glycoprotein, gp120 and/or gp41 under conditions suitable for the modulating of the HIV cell fusion activity.

In one embodiment, a nucleic acid molecule of the invention, for example an aptamer or enzymatic nucleic acid molecule, is chemically synthesized. In another embodiment, the nucleic acid molecule of the invention comprises at least one nucleic acid sugar modification. In yet another embodiment, the nucleic acid molecule of the invention comprises at least one nucleic acid base modification. In another embodiment,

the nucleic acid molecule of the invention comprises at least one nucleic acid backbone modification.

In one embodiment, the nucleic acid molecule of the invention comprises one or more ribonucleotides. In another embodiment, the nucleic acid molecule of the invention 5 comprises one or more deoxy ribonucleotides.

In another embodiment, the nucleic acid molecule of the invention comprises at least one 2'-O-alkyl, 2'-alkyl, 2'-alkoxylalkyl, 2'-alkylthioalkyl, 2'-amino, 2'-O-amino, or 2'-halo modification and/or any combination thereof with or without 2'-deoxy and/or 10 2'-ribo nucleotides. In yet another embodiment, the nucleic acid molecule of the invention comprises all 2'-O-alkyl nucleotides, for example, all 2'-O-allyl nucleotides.

In one embodiment, the nucleic acid molecule of the invention comprises a 5'-cap, 3'-cap, or 5'-3' cap structure, for example, an abasic or inverted abasic moiety.

In another embodiment, the nucleic acid molecule of the invention is a linear nucleic acid molecule. In another embodiment, the nucleic acid molecule of the invention 15 is a linear nucleic acid molecule that can optionally form a hairpin, loop, stem-loop, or other secondary structure. In yet another embodiment, the nucleic acid molecule of the invention is a circular nucleic acid molecule.

In one embodiment, the nucleic acid molecule of the invention is a single stranded oligonucleotide. In another embodiment, the nucleic acid molecule of the invention is a 20 double-stranded oligonucleotide.

In one embodiment, the nucleic acid molecule of the invention comprises an oligonucleotide having about 3 to about 500 nucleotides. In another embodiment, the nucleic acid molecule of the invention comprises an oligonucleotide having about 3 to about 24 nucleotides. In another embodiment, the nucleic acid molecule of the invention 25 comprises an oligonucleotide having about 4 to about 16 nucleotides.

In one embodiment, the nucleic acid aptamer of the invention binds to its corresponding HIV-1 envelope derived target, with a binding affinity of about 100 pM-100 nM or about 20 to 50 nM, for example, by non-covalent interaction of the nucleic acid aptamer with a gp41 or gp120 derived peptide sequence, secondary or tertiary

structure. In another embodiment, the nucleic acid aptamer of the invention binds to the HIV-1 envelope glycoprotein target with a binding affinity of less than about 20 nM.

In another embodiment, the nucleic acid aptamer of the invention binds irreversibly to the HIV-1 envelope derived target, for example, by covalent attachment of the nucleic acid aptamer to gp41 or gp120, or a gp41 or gp120 derived peptide sequence, secondary or tertiary structure. The covalent attachment can be accomplished by introducing chemical modifications into the nucleic acid aptamer's sequence that are capable of forming covalent bonds to the HIV-1 envelope glycoprotein target sequence.

In one embodiment, the invention features a composition comprising at least one HIV reverse transcriptase inhibitor and a nucleic acid molecule of the invention in a pharmaceutically acceptable carrier. In another embodiment, the invention features a composition comprising at least one HIV protease inhibitor and a nucleic acid molecule of the invention in a pharmaceutically acceptable carrier. In yet another embodiment, the invention features a composition comprising at least one HIV reverse transcriptase inhibitor, at least one HIV protease inhibitor and a nucleic acid molecule of the invention in a pharmaceutically acceptable carrier.

In another embodiment, the invention features a method of administering to a cell, for example a mammalian cell or human cell, a nucleic acid molecule of the invention independently or in conjunction with other therapeutic compounds such as HIV reverse transcriptase inhibitors and/or HIV protease inhibitors, comprising contacting the cell with the nucleic acid molecule and the HIV reverse transcriptase inhibitors and/or HIV protease inhibitors under conditions suitable for the administration.

In yet another embodiment, the invention features a method of administering to a cell, for example, a mammalian cell or human cell, a nucleic acid molecule of the invention independently or in conjunction with other therapeutic compounds, such as enzymatic nucleic acid molecules, antisense molecules, triplex forming oligonucleotides, 2,5-A chimeras, and/or RNAi molecules, comprising contacting the cell with the nucleic acid molecule of the invention under conditions suitable for the administration.

In another embodiment, administration of a nucleic acid molecule of the invention is administered to a cell or patient in the presence of a delivery reagent, for example a lipid, cationic lipid, phospholipid, or liposome.

In one embodiment, the invention features a method for identifying nucleic acid aptamers having HIV anti-fusogenic properties comprising: (a) generating a randomized pool of oligonucleotides; (b) combining the oligonucleotides from (a) with gp41 in vitro under conditions suitable to allow at least one oligonucleotide to bind to the target gp41 peptide; (c) removing non-bound oligonucleotide sequences from (b) under conditions suitable for isolating oligonucleotide sequences from (b) that possess binding affinity to gp41 by removing non-bound oligonucleotide sequences; (d) amplifying the oligonucleotide sequences isolated from (c) under conditions suitable for introducing some degree of mutation into the sequences; and (e) repeating steps (c) and (d) under conditions suitable for isolating one or more nucleic acid aptamers having binding affinity to gp41.

In another embodiment, the invention features a method for identifying nucleic acid aptamers having HIV anti-fusogenic properties comprising: (a) generating a randomized pool of oligonucleotides; (b) combining the oligonucleotides from (a) with gp120 in vitro under conditions suitable to allow at least one oligonucleotide to bind to the target gp120 peptide; (c) isolating oligonucleotide sequences from (b) that possess binding affinity to gp120 by removing non-bound oligonucleotide sequences; (d) amplifying the oligonucleotide sequences isolated from (c) under conditions suitable for introducing some degree of mutation into the sequences; and (e) repeating steps (c) and (d) under conditions suitable for isolating one or more nucleic acid aptamers having binding affinity to gp210.

In one embodiment, the invention features a method for identifying nucleic acid aptamers having HIV anti-fusogenic properties comprising: (a) generating a randomized pool of oligonucleotides; (b) combining the oligonucleotides from (a) with a target peptide derived from the HIV envelope glycoprotein in vitro under conditions suitable to allow at least one oligonucleotide to bind to the target peptide; (c) isolating oligonucleotide sequences from (b) that possess binding affinity to the target peptide by removing non-bound oligonucleotide sequences; (d) amplifying the oligonucleotide

sequences isolated from (c) under conditions suitable for introducing some degree of mutation into the sequences; and (e) repeating steps (c) and (d) under conditions suitable for isolating one or more nucleic acid aptamers having binding affinity to the target peptide. In the described methods, the random pool of oligonucleotides can comprise 5 DNA and/or RNA, with or without chemically modified nucleotides. When chemically modified nucleotides are used in the method, such modifications can be chosen such that a non-discriminatory polymerase will incorporate the chemically modified nucleotide into the oligonucleotide sequence when generated or amplified. Non-limiting examples of chemically modified nucleoside triphosphates (NTPs) that can be used in the method of 10 the invention include 2'-deoxy-2'-fluoro, 2'-deoxy-2'-amino, 2'-O-alkyl, and 2'-O-methyl NTPs as well as various base modified NTPs, such as C5-modified pyrimidines, 15 2,6-diaminopurine, and inosine. The oligonucleotides used in the method can be of fixed or variable length. The target peptide derived from HIV envelope glycoprotein used in the method of the invention can comprise a synthetic or naturally occurring peptide that is synthesized or isolated from viral protein, for example by proteolytic cleavage. The 20 target peptide can comprise sequence derived from proteins having sequence identical or similar to GenBank Accession Nos. AAM09869-AAM09880 or analogs thereof. For example, the target peptide can comprise sequences derived from gp41 or gp120 that are essential for HIV membrane fusion and viral entry activity, such as SEQ ID NOs. 1233 and/or 1234, and analogs thereof. These analogs can contain one or more amino acid 25 truncations, deletions, insertions or substitutions. The conditions used in the method preferably provide nucleic acid aptamers that bind to their respective target in the conformation that the target adopts in its natural state. For example, peptide targets and binding conditions are chosen such that the isolated aptamer binds to its target site within the HIV envelope glycoprotein such that fusogenic activity of the protein is disrupted, such as by preventing intermolecular or intramolecular protein-protein interactions. The 30 nucleic acid aptamers thus isolated by methods of the invention can be tested, for example, for an ability to inhibit cell fusion or viral activity using assays described herein.

In another embodiment, the method for identifying nucleic acid aptamers having 30 HIV anti-fusogenic properties comprises attaching the target protein or peptide sequence to a solid matrix, such as beads, microtiter plate wells, membranes, or chip surfaces. In such a system, the target protein/peptide can be attached to the solid matrix either

covalently or non-covalently. In yet another embodiment, the oligonucleotide or nucleic acid aptamer used in a method of the invention can be labeled, either directly or non-directly, for example with a radioactive label, absorption label such as biotin, or a fluorescent label such as fluorescein or rhodamine.

In one embodiment, the invention features novel nucleic acid-based techniques such as nucleic acid aptamers, used alone or in combination with enzymatic nucleic acid molecules, antisense molecules, and/or RNAi molecules, and methods for use to prevent HIV cellular fusion and entry or to down regulate or modulate the expression of HIV RNA and/or replication of HIV.

In another embodiment, the invention features the use of one or more nucleic acid-based techniques, such as nucleic acid aptamers, enzymatic nucleic acid molecules, small interfering RNA (siRNA), nucleic acid sensor molecules, allozymes, antisense nucleic acid molecules, 2,5-A nucleic acid chimeras, triplex oligonucleotides, and antisense nucleic acid molecules with nucleic acid cleaving groups, to modulate the activity, expression, or level of cellular proteins required for HIV cell fusion and entry. For example, the invention features the use of nucleic acid-based techniques to specifically modulate the activity and/or expression of proteins required for HIV cell fusion and entry, such as cellular receptors, cell surface molecules, cellular enzymes, cellular transcription factors, and/or cytokines, second messengers, and cellular accessory molecules.

20 Examples of such cellular receptors involved in HIV infection contemplated by the  
instant invention include, but are not limited to, CD4 receptors, CXCR4 (also known as  
Fusin; LESTR; NPY3R, e.g., Genbank Accession No. NM\_003467); CCR5 (also known  
as CKR-5, CMKRB5, e.g., Genbank Accession No. NM\_000579); CCR3 (also known as  
as CKR-3, CKR-3, CMKBR3, e.g., Genbank Accession No. NM\_001837); CCR2 (also  
known as CKR-2, CMKBR2, e.g., Genbank Accession Nos. NM\_000647 and  
25 NM\_000648); CCR1 (also known as CKR1, CMKBR1, e.g., Genbank Accession No.  
NM\_001295); CCR4 (also known as CKR-4, e.g., Genbank Accession No. NM\_005508);  
CCR8 (also known as ChemR1, TER1, CMKBR8, e.g., Genbank Accession No.  
NM\_005201); CCR9 (also known as D6, e.g. Genbank Accession Nos. NM\_006641 and  
30 NM\_005201); CXCR2 (also known as IL-8RB, e.g., Genbank Accession No.  
NM\_001557); STRL33 (also known as Bonzo; TYMSTR, e.g., Genbank Accession No.

NM\_006564); US28; V28 (also known as CMKBRL1, CX3CR1, GPR13, e.g., Genbank Accession No. NM\_001337); gpr1 (also known as GPR1, e.g., Genbank Accession No. NM\_005279); gpr15 (also known as BOB, GPR15, e.g., Genbank Accession No. NM\_005290); Apj (also known as angiotensin-receptor-like, AGTRL1, e.g., Genbank Accession No. NM\_005161); and ChemR23 receptors (e.g., Genbank Accession No. NM\_004072).

5 Examples of cell surface molecules involved in HIV infection contemplated by the instant invention include, but are not limited to, Heparan Sulfate Proteoglycans, HSPG2 (e.g., Genbank Accession No. NM\_005529); SDC2 (e.g., Genbank Accession Nos. 10 AK025488, J04621, J04621); SDC4 (e.g., Genbank Accession No. NM\_002999); GPC1 (e.g., Genbank Accession No. NM\_002081); SDC3 (e.g., Genbank Accession No. NM\_014654); SDC1 (e.g., Genbank Accession No. NM\_002997); Galactoceramides (e.g., Genbank Accession Nos. NM\_000153, NM\_003360, NM\_001478.2, NM\_004775, and NM\_004861); and Erythrocyte-expressed Glycolipids (e.g., Genbank Accession Nos. 15 NM\_003778, NM\_003779, NM\_003780, NM\_030587, and NM\_001497).

Examples of cellular enzymes involved in HIV infection contemplated by the invention include, but are not limited to, N-myristoyltransferase (NMT1, e.g., Genbank Accession No. NM\_021079 and NMT2, e.g., Genbank Accession No. NM\_004808); Glycosylation Enzymes (e.g., Genbank Accession Nos. NM\_000303, NM\_013339, 20 NM\_003358, NM\_005787, NM\_002408, NM\_002676, NM\_002435), NM\_002409, NM\_006122, NM\_002372, NM\_006699, NM\_005907, NM\_004479, NM\_000150, NM\_005216 and NM\_005668); gp-160 Processing Enzymes (such as PCSK5, e.g., Genbank Accession No. NM\_006200); Ribonucleotide Reductase (e.g., Genbank Accession Nos. NM\_001034, NM\_001033, AB036063, AB036063, AB036532, 25 AK001965, AK001965, AK023605, AL137348, and AL137348); and Polyamine Biosynthesis enzymes (e.g., Genbank Accession Nos. NM\_002539, NM\_003132 and NM\_001634).

30 Examples of cellular transcription factors involved in HIV infection contemplated by the invention include, but are not limited to, SP-1 and NF-kappa B (such as NFKB2, e.g., Genbank Accession No. NM\_002502; RELA, e.g., Genbank Accession No. NM\_021975; and NFKB1, e.g., Genbank Accession No. NM\_003998).

Examples of cytokines and second messengers involved in HIV infection contemplated by the invention include, but are not limited to, Tumor Necrosis Factor-a (TNF-a, e.g., Genbank Accession No. NM\_000594); Interleukin 1a (IL-1a, e.g., Genbank Accession No. NM\_000575); Interleukin 6 (IL-6, e.g., Genbank Accession No. NM\_000600); Phospholipase C (PLC, e.g., Genbank Accession No. NM\_000933); and Protein Kinase C (PKC, e.g., Genbank Accession No. NM\_006255).

Examples of cellular accessory molecules involved in HIV infection contemplated by the invention include, but are not limited to, Cyclophilins, (such as PPID, e.g., Genbank Accession No. NM\_005038; PPIA, e.g., Genbank Accession No. NM\_021130; PPIE, e.g., Genbank Accession No. NM\_006112; PPIB, e.g., Genbank Accession No. NM\_000942; PPIF, e.g., Genbank Accession No. NM\_005729; PPIG, e.g., Genbank Accession No. NM\_004792; and PPIC, e.g., Genbank Accession No. NM\_000943); Mitogen Activated Protein Kinase (MAP-Kinase, such as MAPK1, e.g., Genbank Accession Nos. NM\_002745 and NM\_138957); and Extracellular Signal-Regulated Kinase (ERK-Kinase). In one embodiment, nucleic acid molecules of the invention are used to treat HIV-infected cells or a HIV-infected patient wherein the HIV is resistant or the patient does not respond to treatment with current antiviral therapeutics such as HIV reverse transcriptase or HIV protease inhibitors, either alone or in combination with other therapies under conditions suitable for the treatment.

The present invention also features nucleic acid molecules capable of modulating gene expression, such as enzymatic nucleic acid molecules, small interfering RNA (siRNA), nucleic acid sensor molecules, allozymes, antisense nucleic acid molecules, 2,5-A nucleic acid chimeras, triplex oligonucleotides, and antisense nucleic acid molecules with nucleic acid cleaving groups, which down regulate expression of a sequence encoding a human immunodeficiency virus (such as HIV-1, HIV-2, and related viruses such as FIV-1 and SIV-1) envelope glycoprotein gene (env), for example Genbank accession number NC\_001802 and/or sequences referred to in **Table I**. The sequence descriptions in **Table I** refer to composite names consisting of the following four parts: (a) HIV subtype (A, B, C, etc.); (b) Country of origin (US, JP, etc.); (c) Sampling year (2 digits, a "-" means the sampling year isn't entered); and (d) Sequence name or isolate name.

The present invention features an enzymatic nucleic acid molecule comprising SEQ ID NOs. 505-905. The invention also features an enzymatic nucleic acid molecule comprising at least one binding arm wherein one or more of said binding arms comprises a sequence complementary to any of SEQ ID NOs. 1-395.

5 In one embodiment, an enzymatic nucleic acid molecule of the invention is adapted to HIV infection or acquired immunodeficiency syndrome (AIDS).

In another embodiment, the enzymatic nucleic acid molecule of the invention has an endonuclease activity to cleave RNA having HIV env sequence.

10 In one embodiment, the enzymatic nucleic acid molecule of the invention is in an Inozyme, Zinzyme, G-cleaver, Amberzyme, DNAzyme Hairpin or Hammerhead configuration.

15 In one embodiment, an enzymatic nucleic acid molecule of the invention comprises between 12 and 100 bases complementary to a RNA sequence encoding HIV env. In another embodiment, an enzymatic nucleic acid molecule of the invention comprises between 14 and 24 bases complementary to a RNA sequence encoding HIV env.

In one embodiment, the Hammerhead of the invention comprises a sequence selected from the group consisting of SEQ ID NOs 505-561.

In one embodiment, the Inozyme of the invention comprises a sequence selected from the group consisting of SEQ ID NOs. 562-637.

20 In one embodiment, the G-cleaver of the invention comprises a sequence selected from the group consisting of SEQ ID NOs. 638-661. In one embodiment, the Zinzyme of the invention comprises a sequence selected from the group consisting of SEQ ID NOs. 662-705.

25 In one embodiment, the DNAzyme of the invention comprises a sequence selected from the group consisting of SEQ ID NOs. 706-806.

In one embodiment, the Amberzyme of the invention comprises a sequence selected from the group consisting of SEQ ID NOs 807-905.

In one embodiment, the antisense molecule of the invention comprises a sequence complementary to a sequence of SEQ ID NOS. 1-395. In another embodiment, the antisense molecule of the invention comprises a sequence selected from the group consisting of SEQ ID Nos. 906-1014.

5 In one embodiment, the siRNA molecule of the invention comprises a sequence complementary to a sequence of SEQ ID NOS. 1-395. In another embodiment, the siRNA molecule of the invention comprises a duplex of sequences selected from the group consisting of SEQ ID Nos. 1015-1232.

10 In another embodiment, a nucleic acid molecule of the invention is chemically synthesized. A nucleic acid molecule of the invention can comprise at least one 2'-sugar modification, at least one nucleic acid base modification, and/or at least one phosphate backbone modification.

15 In one embodiment the present invention features a mammalian cell comprising a nucleic acid molecule of the invention. In one embodiment, the mammalian cell of the invention is a human cell.

The invention features a method of reducing HIV activity in a cell comprising contacting the cell with a nucleic acid molecule of the invention under conditions suitable for the reduction of HIV activity.

20 The invention also features a method of treating a patient having a condition associated with the level of HIV comprising contacting cells of the patient with a nucleic acid molecule of the invention under conditions suitable for the treatment.

In one embodiment, methods of treatment contemplated by the invention comprise the use of one or more drug therapies under conditions suitable for the treatment.

25 The invention features a method of cleaving RNA of a HIV env gene comprising contacting a nucleic acid molecule of the invention with the RNA of HIV env gene under conditions suitable for the cleavage. In one embodiment, the cleavage contemplated by the invention is carried out in the presence of a divalent cation, for example Mg<sup>2+</sup>.

In another embodiment, the nucleic acid molecule of the invention comprises a cap structure, wherein the cap structure is at the 5'-end, or 3'-end, or both the 5'-end and the 3'-end of the enzymatic nucleic acid molecule, for example, a 3',3'-linked or 5',5'-linked deoxyabasic ribose derivative.

5 The present invention features an expression vector comprising a nucleic acid sequence encoding at least one nucleic acid molecule of the invention in a manner which allows expression of the nucleic acid molecule.

The invention also features a mammalian cell, for example, a human cell comprising an expression vector contemplated by the invention.

10 In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more nucleic acid molecules, which may be the same or different.

15 The present invention features a method for treatment of acquired immunodeficiency syndrome (AIDS) or an AIDS related condition, for example Kaposi's sarcoma, lymphoma, cervical cancer, squamous cell carcinoma, cardiac myopathy, rheumatic disease, or opportunistic infection, comprising administering to a patient a nucleic acid molecule of the invention under conditions suitable for the treatment.

20 In one embodiment, a nucleic acid molecule of the invention comprises at least five ribose residues, at least ten 2'-O-methyl modifications, and a 3'-end modification, for example, a 3'-3' inverted abasic moiety.

In another embodiment, a nucleic acid molecule of the invention further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides.

25 In yet another embodiment, a DNAzyme of the invention comprises at least ten 2'-O-methyl modifications and a 3'-end modification, for example a 3'-3' inverted abasic moiety. In a further embodiment, the DNAzyme of the invention further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides.

In another embodiment, other drug therapies of the invention comprise antiviral therapy, monoclonal antibody therapy, chemotherapy, radiation therapy, analgesic therapy, or anti-inflammatory therapy.

In yet another embodiment, antiviral therapy of the invention comprises treatment 5 with zidovudine (AZT), zalcitabine (DDC), zidovudine (ZDV), lamivudine (3TC), didanosine/delavirdine (DDI), stavudine (D4T), abacavir, efavirenz, nevirapine, or tenofovir disoproxil fumarate, ribavirin and/or protease inhibitors such as indinavir, amprenavir, saquinavir, lopinavir, ritonavir, or nelfinavir, or any combination thereof.

The invention features a composition comprising a nucleic acid molecule of the 10 invention in a pharmaceutically acceptable carrier.

In one embodiment, the invention features a method of administering to a cell, for example a mammalian cell or human cell, a nucleic acid molecule of the invention comprising contacting the cell with the nucleic acid molecule under conditions suitable for the administration. The method of administration can be in the presence of a delivery 15 reagent, for example, a lipid, cationic lipid, phospholipid, or liposome.

The term "antifusogenic" as used herein refers to the ability of a compound to inhibit or reduce the level of membrane fusion events between two or more moieties relative to the level of membrane fusion which occurs between the moieties in the absence of the compound. The moieties can be, for example, cell membranes or viral 20 structures, such as viral envelopes or pili. Antifusogenic compounds can exert their effect by modulating protein-protein interactions or by modulating intracellular events involving coiled-coil peptide structures.

The term "antiviral" as used herein refers to the ability of a compound to inhibit or reduce viral infection of cells, for example, by inhibiting cell-cell fusion or free virus 25 infection. The antiviral activity of the compound can result from antifusogenic activity or by preventing viral replication and/or expression, such as by modulating the expression of the viral genome.

The term "modulate" as used herein refers to a stimulatory or inhibitory effect on the intracellular or intercellular process of interest relative to the level or activity of such

a process in the absense of a nucleic acid molecule of the invention. For example, the level of membrane fusion events between two or more moieties is enhanced or decreased in the presence of a modulator relative to the level of membrane fusion which occurs between the moieties in the absence of the modulator. In another non-limiting example, 5 the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited 10 to this definition.

The term "inhibit" as used herein refers to when the activity of HIV envelope glycoprotein, or level of RNAs or equivalent RNAs encoding one or more protein subunits of HIV envelope glycoprotein or functional equivalents thereof, is reduced below that observed in the absence of the nucleic acid of the invention. In one 15 embodiment, inhibition with nucleic acid molecule preferably is below that level observed in the presence of non-binding or an inactive or attenuated molecule that is unable to bind to the same target site. In another embodiment, inhibition of HIV gene expression, cell fusion or cell entry with the nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence.

20 The methods of this invention can be used to treat HIV infections, which include productive virus infection, latent or persistent virus infection. The utility can be extended to other species of HIV that infect non-human animals where such infections are of veterinary importance.

25 By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. The aptamer can also be used to prevent protein-protein interactions or 30 conformational changes within a protein by binding to a portion of a target protein that interacts with another protein or with another portion of the same protein. This is a non-limiting example and those in the art will recognize that other embodiments can be

readily generated using techniques generally known in the art, see for example Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628.

5 By "enzymatic nucleic acid molecule" is meant a nucleic acid molecule that has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave a target RNA or DNA molecule. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave a RNA or DNA molecule and thereby inactivate a target RNA or DNA molecule. These 10 complementary regions allow sufficient hybridization of the enzymatic nucleic acid molecule to a target RNA molecule and thus permit cleavage. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention (see for example Werner and Uhlenbeck, 1995, *Nucleic Acids Research*, 23, 2092-2096; Hammann *et al.*, 1999, *Antisense and Nucleic Acid Drug Dev.*, 9, 25-31). 15 The nucleic acids can be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, aptazyme or aptamer-binding ribozyme, regulatable ribozyme, catalytic oligonucleotides, nucleozyme, DNAzyme, RNA enzyme, endoribonuclease, endonuclease, minizyme, leadzyme, oligozyme or DNA enzyme. All 20 of these terminologies describe nucleic acid molecules with enzymatic activity. The specific enzymatic nucleic acid molecules described in the instant application are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it have a specific substrate binding site which is complementary to one or more of the target nucleic acid 25 regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving activity to the molecule (Cech *et al.*, U.S. Patent No. 4,987,071; Cech *et al.*, 1988, *JAMA* 260:20 3030-4).

30 By "nucleic acid molecule" as used herein is meant a molecule comprising nucleotides. The nucleic acid can be single, double, or multiple stranded and can comprise modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof.

By "Inozyme" or "NCH" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described as NCH Rz in Ludwig *et al.*, International PCT Publication No. WO 98/58058 and US Patent Application Serial No. 08/878,640, which is herein incorporated by reference in its entirety including the drawings. Inozymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet NCH/, where N is a nucleotide, C is cytidine and H is adenosine, uridine or cytidine, and / represents the cleavage site. Inozymes can also possess endonuclease activity to cleave RNA substrates having a cleavage triplet NCN/, where N is a nucleotide, C is cytidine, and / represents the cleavage site

By "G-cleaver" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in Eckstein *et al.*, US 6,127,173, which is herein incorporated by reference in its entirety including the drawings, and in Kore *et al.*, 1998, *Nucleic Acids Research* 26, 4116-4120. G-cleavers possess endonuclease activity to cleave RNA substrates having a cleavage triplet NYN/, where N is a nucleotide, Y is uridine or cytidine and / represents the cleavage site. G-cleavers can be chemically modified.

By "zinzyme" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in Beigelman *et al.*, International PCT publication No. WO 99/55857 and US Patent Application Serial No. 09/918,728, which is herein incorporated by reference in its entirety including the drawings. Zinzymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet including but not limited to, YG/Y, where Y is uridine or cytidine, and G is guanosine and / represents the cleavage site. Zinzymes can be chemically modified to increase nuclease stability through various substitutions, including substituting 2'-O-methyl guanosine nucleotides for guanosine nucleotides. In addition, differing nucleotide and/or non-nucleotide linkers can be used to substitute the 5'-gaaa-2' loop of the motif. Zinzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

By "amberzyme" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in Beigelman *et al.*, International PCT publication No. WO 99/55857 and US Patent Application Serial No. 09/476,387,

which is herein incorporated by reference in its entirety including the drawings. Amberzymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet NG/N, where N is a nucleotide, G is guanosine, and / represents the cleavage site. Amberzymes can be chemically modified to increase nuclease stability. In addition, 5 differing nucleoside and/or non-nucleoside linkers can be used to substitute the 5'-gaaa-3' loops of the motif. Amberzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

By 'DNAzyme' is meant, an enzymatic nucleic acid molecule that does not require 10 the presence of a 2'-OH group within its own nucleic acid sequence for activity. In particular embodiments, the enzymatic nucleic acid molecule can have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. DNAzymes can be synthesized chemically or expressed endogenously *in vivo*, by means of a single stranded DNA vector or equivalent 15 thereof. Non-limiting examples of DNAzymes are generally reviewed in Usman *et al.*, US patent No. 6,159,714, which is herein incorporated by reference in its entirety including the drawings; Chartrand *et al.*, 1995, *NAR* 23, 4092; Breaker *et al.*, 1995, *Chem. Bio.* 2, 655; Santoro *et al.*, 1997, *PNAS* 94, 4262; Breaker, 1999, *Nature Biotechnology*, 17, 422-423; and Santoro *et. al.*, 2000, *J. Am. Chem. Soc.*, 122, 2433-39. The "10-23" 20 DNAzyme motif is one particular type of DNAzyme that was evolved using *in vitro* selection as generally described in Joyce *et al.*, US 5,807,718 and Santoro *et al.*, *supra*. Additional DNAzyme motifs can be selected for using techniques similar to those described in these references, and hence, are within the scope of the present invention.

By "nucleic acid sensor molecule" or "allozyme" as used herein is meant a nucleic 25 acid molecule comprising an enzymatic domain and a sensor domain, where the ability of the enzymatic nucleic acid domain's ability to catalyze a chemical reaction is dependent on the interaction with a target signaling molecule, such as a nucleic acid, polynucleotide, oligonucleotide, peptide, polypeptide, or protein, for example HIV-1 envelope glycoprotein, gp41, or gp120. The introduction of chemical modifications, additional 30 functional groups, and/or linkers, to the nucleic acid sensor molecule can provide enhanced catalytic activity of the nucleic acid sensor molecule, increased binding affinity of the sensor domain to a target nucleic acid, and/or improved nuclease/chemical stability

of the nucleic acid sensor molecule, and are hence within the scope of the present invention (see for example Usman *et al.*, US Patent Application No. 09/877,526, George *et al.*, US Patent Nos. 5,834,186 and 5,741,679, Shih *et al.*, US Patent No. 5,589,332, Nathan *et al.*, US Patent No 5,871,914, Nathan and Ellington, International PCT publication No. WO 00/24931, Breaker *et al.*, International PCT Publication Nos. WO 00/26226 and 98/27104, and Sullenger *et al.*, US Patent Application Serial No. 09/205,520).

By "sensor component" or "sensor domain" of the nucleic acid sensor molecule as used herein is meant, a nucleic acid sequence (e.g., RNA or DNA or analogs thereof) which interacts with a target signaling molecule, for example a nucleic acid sequence in one or more regions of a target nucleic acid molecule or more than one target nucleic acid molecule, and which interaction causes the enzymatic nucleic acid component of the nucleic acid sensor molecule to either catalyze a reaction or stop catalyzing a reaction. In the presence of target signaling molecule of the invention, such as HIV-1 envelope glycoprotein or portions thereof such as gp41 and/or gp120, the ability of the sensor component, for example, to modulate the catalytic activity of the nucleic acid sensor molecule, is modulated or diminished. The sensor component can comprise recognition properties relating to chemical or physical signals capable of modulating the nucleic acid sensor molecule via chemical or physical changes to the structure of the nucleic acid sensor molecule. The sensor component can be derived from a naturally occurring nucleic acid binding sequence, for example, RNAs that bind to other nucleic acid sequences *in vivo*. Alternately, the sensor component can be derived from a nucleic acid molecule (aptamer), which is evolved to bind to a nucleic acid sequence within a target nucleic acid molecule. The sensor component can be covalently linked to the nucleic acid sensor molecule, or can be non-covalently associated. A person skilled in the art will recognize that all that is required is that the sensor component is able to selectively modulate the activity of the nucleic acid sensor molecule to catalyze a reaction.

By "target molecule" or "target signaling molecule" is meant a molecule capable of interacting with a nucleic acid sensor molecule, specifically a sensor domain of a nucleic acid sensor molecule, in a manner that causes the nucleic acid sensor molecule to be active or inactive. The interaction of the signaling agent with a nucleic acid sensor molecule can result in modification of the enzymatic nucleic acid component of the

5 nucleic acid sensor molecule via chemical, physical, topological, or conformational changes to the structure of the molecule, such that the activity of the enzymatic nucleic acid component of the nucleic acid sensor molecule is modulated, for example is activated or deactivated. Signaling agents can comprise target signaling molecules such as macromolecules, ligands, small molecules, metals and ions, nucleic acid molecules including but not limited to RNA and DNA or analogs thereof, proteins, peptides, antibodies, polysaccharides, lipids, sugars, microbial or cellular metabolites, pharmaceuticals, and organic and inorganic molecules in a purified or unpurified form, for example HIV envelope glycoprotein or portions thereof such as gp41, gp120, and/or 10 peptide sequences such as SEQ ID Nos 1233 and 1234 or analogs thereof.

By "sufficient length" is meant a nucleic acid molecule long enough to provide the intended function under the expected condition. For example, a nucleic acid molecule of the invention needs to be of "sufficient length" to provide stable binding to a target site under the expected binding conditions and environment. In another non-limiting 15 example, for the binding arms of an enzymatic nucleic acid, "sufficient length" means that the binding arm sequence is long enough to provide stable binding to a target site under the expected reaction conditions and environment. The binding arms are not so long as to prevent useful turnover of the nucleic acid molecule. By "stably interact" is meant interaction of the oligonucleotides with target, such as a target protein or target 20 nucleic acid (e.g., by forming hydrogen bonds with complementary amino acids or nucleotides in the target under physiological conditions) that is sufficient for the intended purpose (e.g., specific binding to a protein target to disrupt the function of that protein or cleavage of target RNA/DNA by an enzyme).

25 By "homology" is meant the nucleotide sequence of two or more nucleic acid molecules, or the amino acid sequence of two or more proteins, is partially or completely identical.

30 By "antisense nucleic acid", it is meant a non-enzymatic nucleic acid molecule that binds to target RNA by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm *et al.*, 1993 *Nature* 365, 566) interactions and alters the activity of the target RNA (for a review, see Stein and Cheng, 1993 *Science* 261, 1004 and Woolf *et al.*, US patent No. 5,849,902). Typically, antisense molecules are complementary to a

target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule can bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule can bind such that the antisense molecule forms a loop. Thus, the antisense molecule can be complementary to two or more non-contiguous substrate sequences or two or more non-contiguous sequence portions of an antisense molecule can be complementary to a target sequence, or both. For a review of current antisense strategies, see Schmajuk *et al.*, 1999, *J. Biol. Chem.*, 274, 21783-21789, Delihas *et al.*, 1997, *Nature*, 315, 751-753, Stein *et al.*, 1997, *Antisense N. A. Drug Dev.*, 7, 151; Crooke, 2000, *Methods Enzymol.*, 313, 3-45; Crooke, 1998, *Biotech. Genet. Eng. Rev.*, 15, 121-157, Crooke, 1997, *Ad. Pharmacol.*, 40, 1-49. Antisense molecules of the instant invention can include 2-5A antisense chimera molecules. In addition, antisense DNA can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. The antisense oligonucleotides can comprise one or more RNase H activating region that is capable of activating RNase H cleavage of a target RNA. Antisense DNA can be synthesized chemically or expressed via the use of a single stranded DNA expression vector or equivalent thereof.

By "RNase H activating region" is meant a region (generally greater than or equal to 4-25 nucleotides in length, preferably from 5-11 nucleotides in length) of a nucleic acid molecule capable of binding to a target RNA to form a non-covalent complex that is recognized by cellular RNase H enzyme (see for example Arrow *et al.*, US 5,849,902; Arrow *et al.*, US 5,989,912). The RNase H enzyme binds to the nucleic acid molecule-target RNA complex and cleaves the target RNA sequence. The RNase H activating region comprises, for example, phosphodiester, phosphorothioate (for example, at least four of the nucleotides are phosphorothioate substitutions; more specifically, 4-11 of the nucleotides are phosphorothioate substitutions), phosphorodithioate, 5'-thiophosphate, or methylphosphonate backbone chemistry or a combination thereof. In addition to one or more backbone chemistries described above, the RNase H activating region can also comprise a variety of sugar chemistries. For example, the RNase H activating region can comprise deoxyribose, arabino, fluoroarabino or a combination thereof, nucleotide sugar chemistry. Those skilled in the art will recognize that the foregoing are non-limiting examples and that any combination of phosphate, sugar and base chemistry of a nucleic

acid that supports the activity of RNase H enzyme is within the scope of the definition of the RNase H activating region and the instant invention.

By "2-5A antisense chimera" it is meant, an antisense oligonucleotide containing a 5'-phosphorylated 2'-5'-linked adenylate residue. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease, which, in turn, cleaves the target RNA (Torrence *et al.*, 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300).

By "triplex nucleic acid" or "triplex oligonucleotide" it is meant a polynucleotide or oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Formation of such triple helix structure has been shown to modulate transcription of the targeted gene (Duval-Valentin *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 504). Triplex nucleic acid molecules of the invention also include steric blocker nucleic acid molecules that bind to the Enhancer I region of HBV DNA (plus strand and/or minus strand) and prevent translation of HBV genomic DNA.

The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication, for example by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner; see for example Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.*, 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*, 297, 2056-60; McManus *et al.*, 2002, *RNA*, 8, 842-850; Reinhart *et al.*, 2002, *Gene & Dev.*, 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions,

wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where 5 one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19 base pairs); the antisense strand comprises 10 nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense 15 and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion 20 thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion 25 thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the 30 siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-

diphosphate. In certain embodiment, the siNA molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic intercations, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting

example, epigenetic regulation of gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure to alter gene expression (see, for example, Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237).

5 By "gene" it is meant, a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional 10 types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its target or complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., ribozyme cleavage, antisense or triple helix modulation. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.*, 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.*, 1986, *Proc. Nat. Acad. Sci. USA* 83:9373-9377; Turner *et al.*, 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the 15 percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). 20 "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

25 The nucleic acid aptamers that bind to a HIV envelope glycoprotein and therefore inactivate the cellular fusion and entry represent a novel therapeutic approach to treat HIV infection, AIDS and related conditions.

In one embodiment of the present invention, an aptamer nucleic acid molecule of the invention is about 4 to about 50 nucleotides in length, in specific embodiments about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 30 nucleotides in length. In another embodiment, an enzymatic nucleic acid molecule of the invention, e.g., a ribozyme or DNAzyme, is about 13 to about 100 nucleotides in length,

e.g., in specific embodiments about 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, or 38 nucleotides in length. In another embodiment, an antisense nucleic acid molecule, 2,5-A chimera, or triplex oligonucleotide of the invention is about 13 to about 100 nucleotides in length, e.g., in specific embodiments about 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, or 38 nucleotides in length. In another embodiment, a siRNA molecule of the invention is about 18 to about 24 nucleotides in length (such as where each strand of siRNA duplex is about 18 to about 24 nucleotides in length), e.g., in specific embodiments, each strand of the siRNA duplex is about 18, 19, 20, 21, 22, 23, or 24 nucleotides in length. In yet another embodiment, a siRNA molecule of the invention has 2 3'-nucleotide overhangs on each strand of the duplex, for example two thymidine (TT) nucleotide overhangs. In particular embodiments, instead of 100 nucleotides being the upper limit on the length ranges specified above, the upper limit of the length range can be, for example, 30, 40, 50, 60, 70, or 80 nucleotides. Thus, for any of the length ranges, the length range for particular embodiments has lower limit as specified, with an upper limit as specified which is greater than the lower limit. For example, in a particular embodiment, the length range can be 20-50 nucleotides in length. All such ranges are expressly included. Also in particular embodiments, a nucleic acid molecule can have a length which is any of the lengths specified above, for example, 21 nucleotides in length.

Aptamer molecules of the invention are about 4 to about 50 nucleotides in length. Exemplary siRNA molecules of the invention are about 18 to about 24 nucleotides in length for each strand of the siRNA duplex. In an additional example, enzymatic nucleic acid molecules of the invention are preferably about 15 to about 50 nucleotides in length, more preferably about 25 to about 40 nucleotides in length, e.g., 34, 36, or 38 nucleotides in length (for example see Jarvis *et al.*, 1996, *J. Biol. Chem.*, 271, 29107-29112). Exemplary DNAzymes of the invention are preferably about 15 to about 40 nucleotides in length. In one embodiment, exemplary DNAzymes are about 25 to about 35 nucleotides in length, e.g., 29, 30, 31, or 32 nucleotides in length (see for example Santoro *et al.*, 1998, *Biochemistry*, 37, 13330-13342; Chartrand *et al.*, 1995, *Nucleic Acids Research*, 23, 4092-4096). Exemplary antisense molecules of the invention are about 15 to about 75 nucleotides in length. In one embodiment, exemplary antisense molecules are about 20 to about 35 nucleotides in length, e.g., 25, 26, 27, or 28 nucleotides in length (see for example Woolf *et al.*, 1992, *PNAS.*, 89, 7305-7309; Milner *et al.*, 1997, *Nature*

*Biotechnology*, 15, 537-541). Exemplary triplex forming oligonucleotide molecules of the invention are about 10 to about 40 nucleotides in length. In one embodiment, exemplary triplex forming oligonucleotide molecules are about 12 to about 25 nucleotides in length, e.g., 18, 19, 20, or 21 nucleotides in length (see for example Maher *et al.*, 1990, *Biochemistry*, 29, 8820-8826; Strobel and Dervan, 1990, *Science*, 249, 73-75). Those skilled in the art will recognize that all that is required is that the nucleic acid molecule is of length and conformation sufficient and suitable for the nucleic acid molecule to catalyze a reaction contemplated herein. The length of the nucleic acid molecules of the instant invention are not limiting within the general limits stated.

10 In one embodiment, the invention provides a method for producing a class of nucleic acid aptamers which exhibit a high degree of specificity for a HIV envelope glycoprotein such as a site within the gp41 region of HIV envelope glycoprotein. In another embodiment, the invention provides a method for producing a class of nucleic acid based gene modulating agents which exhibit a high degree of specificity for HIV 15 nucleic acid sequences encoding the HIV envelope glycoprotein. For example, the nucleic acid gene modulating molecule is preferably targeted to a highly conserved region of the HIV env gene such that specific treatment of a disease or condition can be provided with either one or several nucleic acid molecules of the invention. Alternately, the nucleic acid aptamer molecule is preferably targeted to a highly conserved region of the 20 HIV envelope glycoprotein such that specific treatment of a disease or condition can be provided with either one or several nucleic acid molecules of the invention. Such nucleic acid molecules can be delivered exogenously to specific tissue or cellular targets as required. Alternatively, the nucleic acid molecules can be expressed from DNA and/or RNA vectors that are delivered to specific cells.

25 As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell).

30 By "HIV envelope glycoprotein" is meant, a protein or a mutant protein derivative thereof, comprising sequence expressed and/or encoded by the HIV env gene. Non-

limiting examples of the HIV envelope glycoprotein are represented by Genbank Accession Nos. AAM09869-AAM09880. HIV envelope glycoproteins contemplated by the invention include gp120 and gp41.

By "highly conserved nucleic acid binding region" is meant an amino acid sequence 5 of one or more regions in a target protein that does not vary significantly from one generation to the other or from one biological system to the other.

The enzymatic nucleic acid-based modulators of HIV fusogenic activity are useful for the prevention of the diseases and conditions including HIV infection, AIDS, and any other diseases or conditions that are related to the levels of HIV in a cell or tissue.

10 By "related to the levels of HIV" is meant that the reduction of HIV fusogenic activity and cell entry and/or gene expression (specifically HIV gene) and thus reduction in the level of the HIV expression in an organism will relieve, to some extent, the symptoms of the disease or condition.

15 The nucleic acid-based modulators of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, infusion pump or stent, with or without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in Tables III - XI. 20 Examples of such nucleic acid molecules consist essentially of sequences defined in the tables.

In another aspect, the invention provides mammalian cells containing one or more nucleic acid molecules and/or expression vectors of this invention. The one or more nucleic acid molecules can independently be targeted to the same or different sites.

25 In another aspect of the invention, nucleic acid molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Nucleic acid expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing

nucleic acid molecules of the invention are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of the nucleic acid molecules of the invention. Such vectors might be repeatedly administered as necessary. Once expressed, the nucleic acid molecules of the invention bind to the target protein, RNA and/or DNA and modulate its function or expression. Delivery of nucleic acid expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell. DNA based nucleic acid molecules of the invention can be expressed via the use of a single stranded DNA intracellular expression vector.

By RNA is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a  $\beta$ -D-ribo-furanose moiety.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to express and/or deliver a desired nucleic acid.

By "patient" or "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which the nucleic acid molecules of the invention can be administered. In one embodiment, a patient is a mammal or mammalian cells. In another embodiment, a patient is a human or human cells.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed herein. For example, to treat a disease or condition associated with the levels of HIV, the nucleic acid molecules can be administered to a patient or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the described molecules, such as aptamers, siRNA, antisense, or enzymatic nucleic acids, can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described

molecules could be used in combination with one or more known therapeutic agents to treat HIV infection and/or AIDS. Such therapeutic agents may include, but are not limited to, reverse transcriptase inhibitors such as zidovudine (AZT), zalcitabine (DDC), zidovudine (ZDV), lamivudine (3TC), didanosine/delavirdine (DDI), stavudine (D4T), abacavir, efavirenz, nevirapine, or tenofovir disoproxil fumarate, ribavirin and/or protease inhibitors such as indinavir, amprenavir, saquinavir, lopinavir, ritonavir, or nelfinavir, or any combination thereof under conditions suitable for said treatment.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

10

### BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** is a schematic design which outlines the steps involved in HIV cell fusion and entry.

**Figure 2** is a schematic design that shows a non-limiting example of inhibition of HIV cell fusion and entry.

15

### DETAILED DESCRIPTION OF THE INVENTION

#### Mechanism of action of Nucleic Acid Molecules of the Invention

Aptamer: Nucleic acid aptamers can be selected to specifically bind to a particular ligand of interest (see for example Gold *et al.*, US 5,567,588 and US 5,475,096, Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; 20 Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628). For example, the use of in vitro selection can be applied to evolve nucleic acid aptamers with binding specificity for HIV envelope glycoprotein gp41, gp120 or to any other portion of HIV that disrupts fusogenic activity of the virus. Nucleic acid aptamers can include chemical modifications and linkers as described herein. Nucleic aptamers of the invention can be double stranded or single stranded and can comprise one distinct nucleic acid sequence or more than one nucleic acid sequences complexed with one another. Aptamer molecules of the invention that bind to HIV envelope glycoprotein, for example

gp41, can modulate the fusogenic activity of HIV and therefore modulate cell entry and infectivity of the virus.

Antisense: Antisense molecules can be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides and primarily function by specifically binding to 5 matching sequences resulting in modulation of peptide synthesis (Wu-Pong, Nov 1994, *BioPharm*, 20-33). The antisense oligonucleotide binds to target RNA by Watson Crick base-pairing and blocks gene expression by preventing ribosomal translation of the bound sequences either by steric blocking or by activating RNase H enzyme. Antisense molecules may also alter protein synthesis by interfering with RNA processing or 10 transport from the nucleus into the cytoplasm (Mukhopadhyay & Roth, 1996, *Crit. Rev. in Oncogenesis* 7, 151-190).

In addition, binding of single stranded DNA to RNA may result in nuclease degradation of the heteroduplex (Wu-Pong, *supra*; Crooke, *supra*). To date, the only 15 backbone modified DNA chemistry which will act as substrates for RNase H are phosphorothioates, phosphorodithioates, and borontrifluoridates. Recently, it has been reported that 2'-arabino and 2'-fluoro arabino- containing oligos can also activate RNase H activity.

A number of antisense molecules have been described that utilize novel 20 configurations of chemically modified nucleotides, secondary structure, and/or RNase H substrate domains (Woolf *et al.*, US 5,989,912; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Hartmann *et al.*, USSN 60/101,174 which was filed 25 on September 21, 1998) all of these are incorporated by reference herein in their entirety.

Antisense DNA can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. Antisense DNA 25 can be chemically synthesized or can be expressed via the use of a single stranded DNA intracellular expression vector or the equivalent thereof.

Triplex Forming Oligonucleotides (TFO): Single stranded oligonucleotide can be 30 designed to bind to genomic DNA in a sequence specific manner. TFOs can be comprised of pyrimidine-rich oligonucleotides which bind DNA helices through Hoogsteen Base-pairing (Wu-Pong, *supra*). In addition, TFOs can be chemically

modified to increase binding affinity to target DNA sequences. The resulting triple helix composed of the DNA sense, DNA antisense, and TFO disrupts RNA synthesis by RNA polymerase. The TFO mechanism can result in gene expression or cell death since binding may be irreversible (Mukhopadhyay & Roth, *supra*)

5        2'-5' Oligoadenylates: The 2-5A system is an interferon-mediated mechanism for RNA degradation found in higher vertebrates (Mitra *et al.*, 1996, *Proc Nat Acad Sci USA* 93, 6780-6785). Two types of enzymes, 2-5A synthetase and RNase L, are required for RNA cleavage. The 2-5A synthetases require double stranded RNA to form 2'-5' oligoadenylates (2-5A). 2-5A then acts as an allosteric effector for utilizing RNase L, 10 which has the ability to cleave single stranded RNA. The ability to form 2-5A structures with double stranded RNA makes this system particularly useful for modulation of viral replication.

15        (2'-5') oligoadenylate structures can be covalently linked to antisense molecules to form chimeric oligonucleotides capable of RNA cleavage (Torrence, *supra*). These molecules putatively bind and activate a 2-5A-dependent RNase, the oligonucleotide/enzyme complex then binds to a target RNA molecule which can then be cleaved by the RNase enzyme. The covalent attachment of 2'-5' oligoadenylate structures is not limited to antisense applications, and can be further elaborated to include attachment to nucleic acid molecules of the instant invention.

20        Enzymatic Nucleic Acid: Several varieties of naturally occurring enzymatic RNAs are presently known (Doherty and Doudna, 2001, *Annu. Rev. Biophys. Biomol. Struct.*, 30, 457-475; Symons, 1994, *Curr. Opin. Struct. Biol.*, 4, 322-30). In addition, several *in vitro* selection (evolution) strategies (Orgel, 1979, *Proc. R. Soc. London*, B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing cleavage and 25 ligation of phosphodiester linkages (Joyce, 1989, *Gene*, 82, 83-87; Beaudry *et al.*, 1992, *Science* 257, 635-641; Joyce, 1992, *Scientific American* 267, 90-97; Breaker *et al.*, 1994, *TIBTECH* 12, 268; Bartel *et al.*, 1993, *Science* 261:1411-1418; Szostak, 1993, *TIBS* 17, 89-93; Kumar *et al.*, 1995, *FASEB J.*, 9, 1183; Breaker, 1996, *Curr. Op. Biotech.*, 7, 442; Santoro *et al.*, 1997, *Proc. Natl. Acad. Sci.*, 94, 4262; Tang *et al.*, 1997, *RNA* 3, 914; 30 Nakamaye & Eckstein, 1994, *supra*; Long & Uhlenbeck, 1994, *supra*; Ishizaka *et al.*, 1995, *supra*; Vaish *et al.*, 1997, *Biochemistry* 36, 6495). Each can catalyze a series of

reactions including the hydrolysis of phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions.

Nucleic acid molecules of this invention can block HIV protein expression, specifically, HIV env protein expression, and can be used to treat disease or diagnose 5 disease associated with the levels of HIV.

The enzymatic nature of an enzymatic nucleic acid has significant advantages, such as the concentration of nucleic acid necessary to affect a therapeutic treatment is low. This advantage reflects the ability of the enzymatic nucleic acid molecule to act enzymatically. Thus, a single enzymatic nucleic acid molecule is able to cleave many 10 molecules of target RNA. In addition, the enzymatic nucleic acid molecule is a highly specific modulator, with the specificity of modulation depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can be chosen to completely eliminate catalytic activity of an enzymatic nucleic acid molecule.

15 Nucleic acid molecules having an endonuclease enzymatic activity are able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence-specific manner. With proper design and construction, such enzymatic nucleic acid molecules can be targeted to any RNA transcript, and efficient cleavage achieved *in vitro* (Zaug *et al.*, 324, *Nature* 429 1986; Uhlenbeck, 1987 *Nature* 328, 596; Kim *et al.*, 84 *Proc. Natl. Acad. Sci. USA* 8788, 1987; Dreyfus, 1988, *Einstein Quart. J. Bio. Med.*, 6, 92; Haseloff 20 and Gerlach, 334 *Nature* 585, 1988; Cech, 260 *JAMA* 3030, 1988; and Jefferies *et al.*, 17 *Nucleic Acids Research* 1371, 1989; Chartrand *et al.*, 1995, *Nucleic Acids Research* 23, 4092; Santoro *et al.*, 1997, *PNAS* 94, 4262).

Because of their sequence specificity, *trans*-cleaving enzymatic nucleic acid 25 molecules show promise as therapeutic agents for human disease (Usman & McSwiggen, 1995 *Ann. Rep. Med. Chem.* 30, 285-294; Christoffersen and Marr, 1995 *J. Med. Chem.* 38, 2023-2037). Enzymatic nucleic acid molecule can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the RNA non-functional and abrogates protein expression from that RNA. In this manner, 30 synthesis of a protein associated with a disease state can be selectively modulated (Warashina *et al.*, 1999, *Chemistry and Biology*, 6, 237-250).

The present invention also features nucleic acid sensor molecules or allozymes having sensor domains comprising nucleic acid decoys and/or aptamers of the invention. Interaction of the nucleic acid sensor molecule's sensor domain with a molecular target, such as HIV gp41 or any other suitable HIV target, can activate or inactivate the enzymatic nucleic acid domain of the nucleic acid sensor molecule, such that the activity of the nucleic acid sensor molecule is modulated in the presence of the target-signaling molecule. The nucleic acid sensor molecule can be designed to be active in the presence of the target molecule or alternately, can be designed to be inactive in the presence of the molecular target. For example, a nucleic acid sensor molecule is designed with a sensor domain comprising an aptamer with binding specificity for HIV gp41. In a non-limiting example, interaction of the HIV gp41 with the sensor domain of the nucleic acid sensor molecule can activate the enzymatic nucleic acid domain of the nucleic acid sensor molecule such that the sensor molecule catalyzes a reaction, for example cleavage of HIV RNA. In this example, the nucleic acid sensor molecule is activated in the presence of HIV gp41, and can be used as a therapeutic to treat HIV infection. Alternately, the reaction can comprise cleavage or ligation of a labeled nucleic acid reporter molecule, providing a useful diagnostic reagent to detect the presence of HIV in a system.

#### Synthesis of Nucleic acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than about 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, decoy nucleic acid molecules, aptamer nucleic acid molecules antisense nucleic acid molecules, enzymatic nucleic acid molecules) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, DNA oligonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO

99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, US patent No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid 5 protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2  $\mu$ mol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides. **Table II** outlines the amounts and the contact times of the 10 reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2  $\mu$ mol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60  $\mu$ L of 0.11 M = 6.6  $\mu$ mol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60  $\mu$ L of 0.25 M = 15  $\mu$ mol) can be used in each coupling cycle of 2'-O-methyl residues 15 relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40  $\mu$ L of 0.11 M = 4.4  $\mu$ mol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40  $\mu$ L of 0.25 M = 10  $\mu$ mol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. 20 Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM  $I_2$ , 49 mM pyridine, 9% water in THF (PERSEPTIVE<sup>TM</sup>). Burdick & Jackson Synthesis Grade 25 acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

30 Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is

washed three times with 1.0 mL of EtOH:MeCN:H<sub>2</sub>O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for normal RNA including certain decoy nucleic acid molecules and enzymatic nucleic acid molecules follows the procedure as described in 5 Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et* 10 *al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 15 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2  $\mu$ mol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. Table II outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2  $\mu$ mol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) 15 with minimal modification to the cycle. A 33-fold excess (60  $\mu$ L of 0.11 M = 6.6  $\mu$ mol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60  $\mu$ L of 0.25 M = 15  $\mu$ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to 20 polymer-bound 5'-hydroxyl. A 66-fold excess (120  $\mu$ L of 0.11 M = 13.2  $\mu$ mol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120  $\mu$ L of 0.25 M = 30  $\mu$ mol) can be used in each coupling cycle of ribo residues 25 relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 30 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I<sub>2</sub>, 49 mM pyridine, 9% water in THF (PERSEPTIVE<sup>TM</sup>). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of

phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide 0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to 5 a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to 10 a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH<sub>4</sub>HCO<sub>3</sub>.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on 15 oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 min. The vial is brought to r.t. TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 min. The sample is cooled at -20 °C and then quenched with 1.5 M NH<sub>4</sub>HCO<sub>3</sub>.

For purification of the trityl-on oligomers, the quenched NH<sub>4</sub>HCO<sub>3</sub> solution is 20 loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 min. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

25 Inactive hammerhead ribozymes or binding attenuated control (BAC) oligonucleotides are synthesized by substituting a U for G<sub>5</sub> and a U for A<sub>14</sub> (numbering from Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, 3252). Similarly, one or more nucleotide substitutions can be introduced in other nucleic acid molecules, such as aptamers, to inactivate the molecule and such molecules can serve as a negative control.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format, all that is important is the ratio of chemicals used in the reaction.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 10 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204).

The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). Nucleic acid molecules of the invention can be purified by gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

20 Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see e.g., Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; 25 Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162; Sproat, US Patent No. 5,334,711; Gold *et al.*, US 6,300,074; and Burgin *et al.*, *supra*; all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that

enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS*, 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, *International Publication* PCT No. WO 92/07065; Perrault *et al.* *Nature*, 1990, 344, 565-568; Pieken *et al.* *Science*, 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, 17, 334-339; Usman *et al.* *International Publication* PCT No. WO 93/15187; Sproat, *US Patent* No. 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*, *International PCT publication* No. WO 97/26270; Beigelman *et al.*, *US Patent* No. 5,716,824; Usman *et al.*, *US patent* No. 5,627,053; Woolf *et al.*, *International PCT Publication* No. WO 98/13526; Thompson *et al.*, *USSN 60/082,404* which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into ribozymes without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the nucleic acid molecules of the instant invention.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorothioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be

minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

Nucleic acid molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets. In another embodiment, nucleic acid molecules of the invention include one or more LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of nucleic acid molecules targeting HIV. Such conjugates and/or complexes can be used to facilitate delivery of molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the

pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, 5 toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid 10 molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, US 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable nucleic acid linker molecule" as used herein, refers to a 15 nucleic acid molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule. The stability of the biodegradable nucleic acid linker molecule can be modulated by using various combinations of ribonucleotides, deoxyribonucleotides, and chemically modified nucleotides, for example, 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O- 20 allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. 25 The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein, refers to compounds or 30 molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active molecules contemplated by the instant

invention include therapeutically active molecules such as antibodies, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siRNA, dsRNA, allozymes, 5 aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule 10 comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules of the invention delivered exogenously 15 optimally are stable within cells such that therapeutic activity is achieved. The nucleic acid molecules can therefore be designed such that they are resistant to nucleases and function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

20 In yet another embodiment, nucleic acid molecules having chemical modifications that maintain or enhance enzymatic activity and/or nuclease stability are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered. As exemplified herein, such nucleic acid molecules are useful *in vitro* and/or *in vivo* even if 25 activity over all is reduced 10 fold (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090).

Use of the nucleic acid-based molecules of the invention will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple nucleic acid molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with 30 combinations of molecules (including different motifs) and/or other chemical or

biological molecules. The treatment of patients with nucleic acid molecules may also include combinations of different types of nucleic acid molecules.

In another aspect the nucleic acid molecules comprise a 5' and/or a 3'- cap structure.

5 By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Wincott *et al.*, WO 97/26270, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples the 5'-cap is selected 10 from inverted abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythofuranosyl) nucleotide; 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco 15 nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate 20 moiety (for more details, see Wincott *et al.*, International PCT publication No. WO 97/26270, incorporated by reference herein).

In another embodiment, the 3'-cap is selected from 4',5'-methylene nucleotide; 1-(beta-D-erythofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-25 aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-30 butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging

methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, 5 including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub> or N(CH<sub>3</sub>)<sub>2</sub>, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub>, halogen, 20 N(CH<sub>3</sub>)<sub>2</sub>, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. 25 When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub> or N(CH<sub>3</sub>)<sub>2</sub>, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, 30 heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH,

cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra*, all are hereby incorporated by reference herein).

There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases may be used at any position, for example, within the catalytic core of a nucleic acid decoy molecule and/or in the substrate-binding regions of the nucleic acid molecule.

In one embodiment, the invention features modified nucleic acids, for example aptamers, siRNA, antisense, and enzymatic nucleic acid molecules with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, morpholino, amide carbamate, carboxymethyl, acetamide, 5 polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties*, in *Modern Synthetic Methods*, VCH, 331-417, and Mesmaeker *et al.*, 1994, *Novel Backbone Replacements for Oligonucleotides*, in *Carbohydrate Modifications in Antisense Research*, ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, (for more details, see Usman *et al.*, US 5,891,683 and Matulic-Adamic *et al.*, US 5,998,203).

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, 15 thymine, uracil joined to the 1' carbon of  $\beta$ -D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate.

In connection with 2'-modified nucleotides as described for the present invention, 20 by "amino" is meant 2'-NH<sub>2</sub> or 2'-O- NH<sub>2</sub>, which may be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.*, U.S. Patent 5,672,695 and Matulic-Adamic *et al.*, WO 98/28317.

Various modifications to nucleic acid (e.g., aptamer, siRNA, antisense and enzymatic nucleic acid) structure can be made to enhance the utility of these molecules. 25 Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such oligonucleotides to the target site, e.g., to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Administration of Nucleic Acid Molecules

Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; 5 Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Sullivan *et al.*, PCT WO 94/02595, further describes the general methods for delivery of enzymatic nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells 10 by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). Alternatively, the nucleic acid/vehicle combination is 15 locally delivered by direct injection or by use of an infusion pump. Direct injection of the nucleic acid molecules of the invention, whether subcutaneous, intramuscular, or intradermal, can take place using standard needle and syringe methodologies, or by needle-free technologies such as those described in Conry *et al.*, 1999, *Clin. Cancer Res.*, 5, 2330-2337 and Barry *et al.*, International PCT Publication No. WO 99/31262. The 20 molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a patient.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and 25 the like. The negatively charged polynucleotides of the invention can be administered (e.g., RNA, DNA or protein) and introduced into a patient by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention may also be 30 formulated and used as tablets, capsules or elixirs for oral administration, suppositories for rectal administration, sterile solutions, suspensions for injectable administration, and the other compositions known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

5 A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic administration, into a cell or patient, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to 10 which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

15 By “systemic administration” is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes expose the desired negatively charged 20 polymers, *e.g.*, nucleic acids, to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the 25 reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach may provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cancer cells.

30 By “pharmaceutically acceptable formulation” is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Nonlimiting examples of agents suitable for formulation with the nucleic acid molecules

of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85), which can enhance entry of drugs into the CNS (Jolliet-Riant and Tillement, 1999, *Fundam. Clin. Pharmacol.*, 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after intracerebral implantation (Emerich, DF *et al.*, 1999, *Cell Transplant.*, 8, 47-58) (Alkermes, Inc. Cambridge, MA); and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (*Prog Neuropsychopharmacol Biol Psychiatry*, 23, 941-949, 1999). Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al.* *Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al.*, *Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al.*, *Science* 1995, 267, 1275-1276; Oku *et al.*, 1995, *Biochim. Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al.*, *J. Biol. Chem.* 1995, 42, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or administration, which include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, 5 for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985) hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents may be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents may be used.

10 A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence of, or treat (alleviate a symptom to some extent, preferably all of the symptoms) a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, 15 concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The present invention also includes compositions prepared for storage or 20 administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, 25 dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) 30 of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the

physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

5        The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (*e.g.*, intravenous), intramuscular, or intrathecal injection or 10 infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical 15 compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such 20 compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium 25 carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known 30 techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

5       Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, 10 lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as 15 polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

20       Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved 25 by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. 30 Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, 5 lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, 10 propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable 15 dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3- 20 butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at 25 ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local 30 anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per patient per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending 5 upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular patient depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate 10 of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate 15 quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention may also be administered to a patient in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication may increase the 20 beneficial effects while reducing the presence of side effects.

In one embodiment, the invention compositions suitable for administering nucleic acid molecules of the invention to specific cell types, such as hepatocytes. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal glycoproteins, such 25 as asialoorosomucoid (ASOR). Binding of such glycoproteins or synthetic glycoconjugates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triantennary structures are bound with greater affinity than biantennary or monoantennary chains (Baenziger and Fiete, 1980, *Cell*, 22, 611-620; Connolly *et al.*, 1982, *J. Biol. Chem.*, 257, 939-945). Lee and 30 Lee, 1987, *Glycoconjugate J.*, 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the

receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-terminating glycoproteins or glycoconjugates (Ponpipom *et al.*, 1981, *J. Med. Chem.*, 24, 1388-1395). The use of galactose and galactosamine based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to the treatment of liver disease such as HBV infection or hepatocellular carcinoma. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavailability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention.

10        Alternatively, certain of the nucleic acid molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, *Science*, 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci., USA* 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Dropulic *et al.*, 1992, *J. Virol.*, 66, 1432-41; Weerasinghe *et al.*, 1991, *J. Virol.*, 65, 5531-4; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science*, 247, 1222-1225; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45; all of these references are hereby incorporated in their totalities by reference herein). Those skilled in the art realize that any nucleic acid can be expressed 15 in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a ribozyme (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. 20 Biol. Chem.*, 269, 25856; all of these references are hereby incorporated in their totality by reference herein).

30        In another aspect of the invention, RNA molecules of the present invention are preferably expressed from transcription units (see, for example, Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the nucleic acid

molecules are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of nucleic acid molecules. Such vectors might be repeatedly administered as necessary. Once expressed, the nucleic acid molecule binds to the target mRNA. Delivery of nucleic acid molecule expressing vectors could be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect, the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the nucleic acid molecules of the instant invention is disclosed. The nucleic acid sequence encoding the nucleic acid molecule of the instant invention is operably linked in a manner which allows expression of that nucleic acid molecule.

In another aspect the invention features an expression vector comprising: a) a transcription initiation region (e.g., eukaryotic pol I, II or III initiation region); b) a transcription termination region (e.g., eukaryotic pol I, II or III termination region); c) a nucleic acid sequence encoding at least one of the nucleic acid catalyst of the instant invention; and wherein said sequence is operably linked to said initiation region and said termination region in a manner which allows expression and/or delivery of said nucleic acid molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the nucleic acid catalyst of the invention; and/or an intron (intervening sequences).

Transcription of the nucleic acid molecule sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*,

1990, *Mol. Cell. Biol.*, 10, 4529-37). All of these references are incorporated by reference herein. Several investigators have demonstrated that nucleic acid molecules, such as ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang et al., 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen et al., 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu et al., 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier et al., 1992, *EMBO J.*, 11, 4411-8; Lisziewicz et al., 1993, *Proc. Natl. Acad. Sci. U. S. A.*, 90, 8000-4; Thompson et al., 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 10 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as ribozymes in cells (Thompson et al., *supra*; Couture and Stinchcomb, 1996, *supra*; Noonberg et al., 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg et al., US Patent No. 5,624,803; Good et al., 1997, *Gene Ther.*, 4, 45; Beigelman et al., International PCT Publication No. WO 15 96/18736; all of these publications are incorporated by reference herein). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 20 1996, *supra*).

In yet another aspect, the invention features an expression vector comprising nucleic acid sequence encoding at least one of the nucleic acid molecules of the invention in a manner that allows expression of that nucleic acid molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription 25 termination region; c) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region and said termination region in a manner which allows expression and/or delivery of said nucleic acid molecule. In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading 30 frame; d) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame and wherein said sequence is operably linked to said initiation region, said open reading frame

and said termination region in a manner which allows expression and/or delivery of said nucleic acid molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) a nucleic acid sequence encoding at least one said nucleic acid molecule and wherein said sequence is operably linked to said initiation region, said intron and said termination region in a manner which allows expression and/or delivery of said nucleic acid molecule. In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; e) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame and wherein said sequence is operably linked to said initiation region, said intron, said open reading frame and said termination region in a manner which allows expression and/or delivery of said nucleic acid molecule.

Examples:

15 The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1: Identification of Aptamers that specifically bind to HIV gp41

20 A nucleic acid aptamer that selectively binds HIV gp41 is provided in accordance with the present invention. The binding affinity of the aptamer for HIV gp41 is preferably represented by the dissociation constant of about 20 nanomolar (nM) or less, and more preferably about 10 nM or less. In one embodiment, the Kd of the aptamer and gp41 target is established using a double filter nitrocellulose filter binding assay such as that disclosed by Wong and Lohman, 1993, *PNAS USA*, 90, 5428-5432.

25 Generally, the method for isolating aptamers of the invention having specificity for HIV gp41 comprises: (a) preparing a candidate mixture of potential oligonucleotide ligands for gp41 wherein the candidate mixture is complex enough to contain at least one oligonucleotide ligand for gp41 or a peptide derivative thereof (the gp41 target); (b) contacting the candidate mixture with the gp41 target under conditions suitable for at least one oligonucleotide in the candidate mixture to bind to the gp41 target; (c) removing 30 unbound oligonucleotides from the candidate mixture; (d) collecting the oligonucleotide

ligands that are bound to the gp41 target to produce a first collected mixture of oligonucleotide ligands; (e) contacting the mixture from (d) with the gp41 target under more stringent binding conditions than in (b), wherein oligonucleotide ligands having increased affinity to the gp41 target relative to the first collected mixture of (d); (f) 5 removing unbound oligonucleotides from (e); and (g) collecting the oligonucleotide ligands that are bound to the gp41 target to produce a second collected mixture of oligonucleotide ligands to thereby identify oligonucleotides having specificity for HIV gp41. The method can comprise additional steps in which the oligonucleotides isolated in the first or second collected mixture are enriched or expanded by any suitable technique, 10 such as amplification or mutagenesis, prior to contacting the first collected oligonucleotide mixture with the target under the higher stringency conditions, after collecting the oligonucleotides that bound to the target under the higher stringency conditions, or both. Optionally, the contacting and expanding or enriching steps are repeated as necessary to produce the desired aptamer. Thus, it is possible that the second 15 collected oligonucleotide mixture can comprise a single aptamer. The conditions used to affect the stringency of binding used in the method can include varying reaction conditions used for binding, for example the composition of a buffer, temperature, time, and concentration of the components used for binding can be optimized for the desired level of stringency.

20 In vitro Selection

In a non-limiting example, aptamers having binding specificity for a HIV-1 gp41 target are isolated by applying the method under the following conditions. First, the gp41 target is attached to a solid matrix such as a bead or chip surface by means of a covalent (eg. amide or morpholino bond) or non-covalent (eg. biotin/streptavidin) linkage. The 25 gp41 target can comprise the entire isolated gp41 subunit of HIV envelope glycoprotein or an isolated peptide sequence derived therefrom, such as a peptide having SEQ ID NOS. 1233 and/or 1234. The isolated peptide sequence can be synthesized or isolated by protein digest.

A random pool of DNA oligomers is synthesized where the 5' and 3' proximal ends 30 are fixed sequences used for amplification and the central region consists of randomized positions. Ten picomoles of template are PCR amplified for 8 cycles in the initial round.

Copy DNA of the selected pool of RNA from subsequent rounds of amplification are PCR amplified 18 cycles. PCR reactions are carried out in a 50 .mu.l volume containing 200 picomoles of each primer, 2 mM final concentration dNTP's, 5 units of *Thermus aquaticus* DNA polymerase (Perkin Elmer Cetus) in a PCR buffer (10 mM Tris-Cl pH 5.4, 50 mM KCl, 7.5 mM MgCl<sub>2</sub>, 0.05 mg/ml BSA). Primers are annealed at 58.degree. C. for 20 seconds and extended at 74.degree. C. for 2 minutes. Denaturation can occur at 93°C for 30 seconds.

Products from PCR amplification are used for T7 *in vitro* transcription in a 200 .mu.l reaction volume. T7 transcripts are purified from an 8 percent, 7M Urea polyacrylamide gel and eluted by crushing gel pieces in a Sodium Acetate/EDTA solution. For each round of amplification, 50 picomoles of the selected pool of RNA is phosphatased for 30 minutes using Calf Intestinal Alkaline Phosphatase. The reaction is then phenol extracted 3 times and chloroform extracted once, then ethanol precipitated. 25 picomoles of this RNA is 5' end-labeled using .gamma. <sup>32</sup>P ATP with T4 polynucleotide kinase for 30 minutes. Kinased RNA is gel purified and a small quantity (about 150 fmoles; 100,000 cpm) is used along with 250 picomoles of cold RNA to follow the fraction of RNA bound to gp41 and retained on nitrocellulose filters during the separation step of the method. Typically a protein concentration is used that binds one to five percent of the total input RNA. A control (without protein) is used to determine the background which is typically 0.1% of the total input. Selected RNA is eluted from the filter by extracting three times with water saturated phenol containing 2% lauryl sulfate (SDS), 0.3M NaOAc and 5 mM EDTA followed by a chloroform extraction. Twenty five percent of this RNA is then used to synthesize cDNA for PCR amplification.

#### Selection with Non-Amplifiable Competitor RNA

25 In a non-limiting example, selections are performed using two buffer conditions where the only difference between the buffers is sodium concentration (250 mM NaCl or 500 mM NaCl). Two different buffer conditions are used to increase stringency (with the higher salt concentration being more stringent) and to determine whether different ligands can be obtained. After 10 rounds of amplification, the binding constant of the selected pool can decrease by about an order of magnitude and can remain constant for the next two additional rounds. Competitor RNA is not used in the first 12 rounds. After this

round, the pool is split and selection carried out in the presence and absence (control) of competitor RNA. For rounds 12 through 18, a 50-fold excess of a non-amplifiable random pool of RNA is present during selection to compete with non-specific low-affinity binders that may survive and thus be amplified. The competitor RNA, which had 5 a 30N random region, is made as described above for the amplifiable pool RNA; however, the competitor RNA has different primer annealing sequences. Thus, the competitor RNA does not survive the cDNA synthesis or PCR amplification steps. It would be apparent to one skilled in the art that other primer sequences could be used as long as they are not homologous to those used for the pool RNA. The use of competitor 10 RNA can increase the affinity of the selected pool by several orders of magnitude.

#### Cloning and Sequencing

In a non-limiting example, PCR amplified DNA from the last round selected-pool of RNA is phenol and chloroform extracted and ethanol precipitated. The extracted PCR DNA is then digested using Bam HI and Hind III restriction enzymes and sub-cloned into 15 pUC18. DNAs are phenol and chloroform extracted following digestion. Ligation is carried out at room temperature for two hours after which time the reaction is phenol and chloroform extracted and used to electroporate competent cells. Fifty transformants from the selections using competitor RNA at both NaCl concentrations are picked and their DNAs sequenced.

#### Binding Assays

In a non-limiting example, binding assays are performed by adding 5 .mu.l of HIV-1 gp41 protein, at the appropriate concentrations (i.e., ranging from  $2 \times 10^{-6}$  with 3 fold dilutions to  $9 \times 10^{-9}$  for 250 mM NaCl and  $0.5 \times 10^{-7}$  with 3 fold dilutions to  $2 \times 10^{-10}$  for 50 mM NaCl), to 45 ul of binding buffer (50 mM Na-HEPES pH 7.5, 250 mM NaCl, 25 2 mM DTT, 10 mM MnCl<sub>2</sub>, 5 mM CHAPS) on ice, then adding 50,000 cpm of kinased RNA (<200 fmole) in a volume of 3 to 4 .mu.l. This mix is incubated at 37°C for 20 minutes. The reactions are then passed over nitrocellulose filters, which are pre-equilibrated in buffer, and washed with a 50 mM Tris-Cl pH 7.5 solution. Filters are dried and counted.

General Considerations in Aptamer Selection

When a consensus sequence is identified, oligonucleotides that contain that sequence can be made by conventional synthetic or recombinant techniques. These aptamers can also function as target-specific aptamers of this invention. Such an aptamer 5 can conserve the entire nucleotide sequence of an isolated aptamer, or can contain one or more additions, deletions or substitutions in the nucleotide sequence, as long as a consensus sequence is conserved. A mixture of such aptamers can also function as target-specific aptamers, wherein the mixture is a set of aptamers with a portion or portions of their nucleotide sequence being random or varying, and a conserved region that contains 10 the consensus sequence. Additionally, secondary aptamers can be synthesized using one or more of the modified bases, sugars and linkages described herein using conventional techniques and those described herein.

In some embodiments of this invention, aptamers can be sequenced or mutagenized 15 to identify consensus regions or domains that are participating in aptamer binding to target, and/or aptamer structure. This information is used for generating second and subsequent pools of aptamers of partially known or predetermined sequence. Sequencing used alone or in combination with the retention and selection processes of this invention, can be used to generate less diverse oligonucleotide pools from which aptamers can be made. Further selection according to these methods can be carried out to generate 20 aptamers having preferred characteristics for diagnostic or therapeutic applications. That is, domains that facilitate, for example, drug delivery could be engineered into the aptamers selected according to this invention.

Although this invention is directed to making aptamers using screening from pools 25 of non-predetermined sequences of oligonucleotides, it also can be used to make second-generation aptamers from pools of known or partially known sequences of oligonucleotides. A pool is considered diverse even if one or both ends of the oligonucleotides comprising it are not identical from one oligonucleotide pool member to another, or if one or both ends of the oligonucleotides comprising the pool are identical 30 with non-identical intermediate regions from one pool member to another. Toward this objective, knowledge of the structure and organization of the target protein can be useful to distinguish features that are important for biochemical pathway inhibition or biological

response generation in the first generation aptamers. Structural features can be considered in generating a second (less random) pool of oligonucleotides for generating second round aptamers:

Those skilled in the art will appreciate that comparisons of the complete or partial 5 amino acid sequences of the purified protein target to identify variable and conserved regions is useful. Comparison of sequences of aptamers made according to this invention provides information about the consensus regions and consensus sequences responsible for binding. It is expected that certain nucleotides will be rigidly specified and certain 10 positions will exclusively require certain bases. Likewise, studying localized regions of a protein to identify secondary structure can be useful. Localized regions of a protein can adopt a number of different conformations including beta strands, alpha helices, turns (induced principally by proline or glycine residues) or random structure. Different regions 15 of a polypeptide interact with each other through hydrophobic and electrostatic interactions and also by formation of salt bridges, disulfide bridges, etc. to form the secondary and tertiary structures. Defined conformations can be formed within the protein organization, including beta sheets, beta barrels, and clusters of alpha helices.

It sometimes is possible to determine the shape of a protein target or portion thereof by crystallography X ray diffraction or by other physical or chemical techniques known to those skilled in the art. Many different computer programs are available for predicting 20 protein secondary and tertiary structure, the most common being those described in Chou and Fasman, 1978, *Biochemistry*, 13, 222-245, and Gamier *et al.*, 1978, *J. Mol. Biol.*, 120, 97-120. Generally, these and other available programs are based on the physical and 25 chemical properties of individual amino acids (hydrophobicity, size, charge and presence of side chains) and on the amino acids' collective tendency to form identifiable structures in proteins whose secondary structure has been determined. Many programs attempt to weight structural data with their known influences. For example, amino acids such as proline or glycine are often present where polypeptides have share turns. Long stretches of hydrophobic amino acids (as determined by hydropathy plot), usually have a strong affinity for lipids.

30 Data obtained by the methods described above and by other conventional methods and tools can be correlated with the presence of particular sequences of nucleotides in the

first and second generation aptamers to engineer second and third generation aptamers. Further, according to this invention, second generation aptamers can be identified simply by sequentially screening from pools of oligonucleotides having more predetermined sequences than the pools used in earlier rounds of selection.

5        These methods can be used to design optimal binding sequences for any desired protein target (which can be portions of aptamers or entire aptamers) and/or to engineer into aptamers any number of desired targeted functions or features. Optimal binding sequences are those which exhibit high relative affinity for target, i.e., affinity measured in  $K_d$  in at least in the nanomolar range, and, for certain drug applications, the nanomolar or picomolar range. In practicing this invention, studying the binding energies of aptamers using standard methods known generally in the art are useful. Generally, consensus regions can be identified by comparing the conservation of nucleotides for appreciable enhancement in binding.

15      Structural knowledge can be used to engineer aptamers made according to this invention. For example, stem structures in the aptamer pool can be vital components in some embodiments where increased aptamer rigidity is desired. According to the teachings of the instant invention, a randomly generated pool of oligonucleotides having the stem sequences can be generated. After aptamers are identified which contain the stem (i.e., use the stem in primers), cross-linkers can be introduced into the stem to 20 covalently fix the stem in the aptamer structure. Cross-linkers also can be used to fix an aptamer to a target. Once an aptamer has been identified, it can be used, either by linkage to, or use in combination with, other aptamers identified according to these methods. One or more aptamers can be used in this manner to bind to one or more targets.

#### Techniques used in optimizing Aptamer binding

25      In order to produce nucleic acid aptamers desirable for use as a pharmaceutical composition, it is desirable that the nucleic acid aptamer have the following characteristics: (1) the nucleic acid aptamer binds to the target in a manner capable of achieving the desired effect on the target; (2) be as small as possible to obtain the desired effect; (3) be as stable as possible; and (4) be a specific ligand to the chosen target. In 30 most, if not all, situations it is preferred that the nucleic acid ligand has the highest possible affinity to the target. Modifications or derivatizations of the ligand that confer

resistance to degradation and clearance *in situ* during therapy, the capability to cross various tissue or cell membrane barriers, or any other accessory properties that do not significantly interfere with affinity for the target molecule can also be provided as improvements.

5 One of the uses of nucleic acid ligands derived by *in vitro* selection or another approach is to find ligands that alter target molecule function. Thus, it is a good procedure to first assay for inhibition or enhancement of function of the target protein. One could even perform such functional tests of the combined ligand pool prior to cloning and sequencing. Assays for the biological function of the chosen target are generally available  
10 and known to those skilled in the art, and can be easily performed in the presence of the nucleic acid ligand to determine if inhibition occurs.

Enrichment can supply a number of cloned ligands of probable variable affinity for the target molecule. Sequence comparisons can yield consensus secondary structures and primary sequences that allow grouping of the ligand sequences into motifs. Although a  
15 single ligand sequence (with some mutations) can be found frequently in the total population of cloned sequences, the degree of representation of a single ligand sequence in the cloned population of ligand sequences cannot absolutely correlate with affinity for the target molecule. Therefore mere abundance is not the sole criterion for judging "winners" after *in vitro* selection and binding assays for various ligand sequences  
20 (adequately defining each motif that is discovered by sequence analysis) are required to weigh the significance of the consensus arrived at by sequence comparisons. The combination of sequence comparison and affinity assays should guide the selection of candidates for more extensive ligand characterization.

An important step for determining the length of sequence relevant to specific  
25 affinity is to establish the boundaries of that information within a ligand sequence. This is conveniently accomplished by selecting end-labeled fragments from hydrolyzed pools of the ligand of interest so that 5' and 3' boundaries of the information can be discovered. To determine a 3' boundary, one can perform a large-scale *in vitro* transcription of the amplified aptamer sequence, gel purify the RNA using UV shadowing on an intensifying  
30 screen, phosphatasing the purified RNA, phenol extracting extensively, labeling by kinase reactions with 32P, and gel purification of the labeled product (for example by using a

film of the gel as a guide). The resultant product can then be subjected to pilot partial digestions with RNase T1 (varying enzyme concentration and time, at 50°C in a buffer of 7M urea, 50 mM sodium citrate pH 5.2) and alkaline hydrolysis (at 50 mM NaC03, adjusted to pH 9.0 by prior mixing of 1 M bicarbonate and carbonate solutions; test over ranges of 20 to 60 minutes at 95°C). Once optimal conditions for alkaline hydrolysis are established (so that there is an even distribution of small to larger fragments) one can scale up to provide enough material for selection by the target (for example on nitrocellulose filters). Binding assays can be set up, which vary target protein concentration from the lowest saturating protein concentration to that protein concentration at which approximately 10% of RNA is bound as determined by the binding assays for the ligand. One can vary target concentration by increasing volume rather than decreasing the absolute amount of target; this provides a good signal to noise ratio as the amount of RNA bound to the filter is limited by the absolute amount of target. The RNA is eluted as, for example, in *in vitro* selection and then run on a denaturing gel with T1 partial digests so that the positions of hydrolysis bands can be related to the ligand sequence.

The 5' boundary can be similarly determined. Large-scale *in vitro* transcriptions are purified as described herein. There are two methods for labeling the 3' end of the RNA. One method is to kinase Cp with 32P (or purchase 32P-Cp) and ligate to the purified RNA with RNA ligase. The labeled RNA is then purified and subjected to very identical protocols. An alternative is to subject unlabeled RNAs to partial alkaline hydrolyses and extend an annealed, labeled primer with reverse transcriptase as the assay for band positions. One of the advantages over pCp labeling is the ease of the procedure, the more complete sequencing ladder (by dideoxy chain termination sequencing) with which one can correlate the boundary, and increased yield of assayable product. A disadvantage is that the extension on eluted RNA sometimes contains artifactual stops, so it can be important to control by spotting and eluting starting material on nitrocellulose filters without washes and assaying as the input RNA. Using techniques as described herein, it is possible to find the boundaries of the sequence information required for high affinity binding to the target.

Assessment of Nucleotide Contributions to Aptamer Target Binding Affinity

Once a minimal high affinity ligand sequence is identified, the sequence can be used to identify the nucleotides within the boundaries that are critical to the interaction with the target molecule. One method is to create a new random template in which all of 5 the nucleotides of a high affinity ligand sequence are partially randomized or blocks of randomness are interspersed with blocks of complete randomness for use in an *in vitro* selection method for example, preferably a modified *in vitro* selection method as disclosed herein. Such "secondary" *in vitro* selections produce a pool of ligand sequences 10 in which critical nucleotides or structures are absolutely conserved, less critical features preferred, and unimportant positions unbiased. Secondary *in vitro* selections can thus help to further elaborate a consensus that is based on relatively few ligand sequences. In addition, even higher-affinity ligands can be provided whose sequences were unexplored 15 in the original *in vitro* selection.

Another method is to test oligo-transcribed variants (i.e. nucleotide substitution) 15 where the consensus sequence can be unclear. An additional useful set of techniques are inclusively described as chemical modification experiments. Such experiments can be used to probe the native structure of RNAs, by comparing modification patterns of denatured and non-denatured states. The chemical modification pattern of an RNA ligand 20 that is subsequently bound by target molecule can be different from the native pattern, indicating potential changes in structure upon binding or protection of groups by the target molecule. In addition, RNA ligands can fail to be bound by the target molecule when modified at positions critical to either the bound structure of the ligand or critical to 25 interaction with the target molecule. Such experiments in which these positions are identified are described as "chemical modification interference" experiments.

25 There are a variety of available reagents to conduct such experiments that are known to those skilled in the art (see for example, Ehresmann *et al.*, 1987, *Nuc. Acids. Res.*, 15, 9109-9128. Chemicals that modify bases can be used to modify ligand RNAs. A pool is bound to the target at varying concentrations and the bound RNAs recovered (such 30 as in the boundary experiments) and the eluted RNAs analyzed for the modification. An assay can be by subsequent modification-dependent base removal and aniline scission at the baseless position or by reverse transcription assay of sensitive (modified) positions. In

such assays, bands (indicating modified bases) in unselected RNAs, appear that disappear relative to other bands in target protein-selected RNAs. Similar chemical modifications with ethyl nitrosourea, or via mixed chemical or enzymatic synthesis with, for example, 2'-methoxys on ribose or phosphorothioates can be used to identify essential atomic groups on the oligonucleotide backbone. In experiments with 2'-methoxy versus 2'-OH mixtures, the presence of an essential OH group can result in enhanced hydrolysis relative to other positions in molecules that have been stringently selected by the target.

Comparisons of the intensity of bands for bound and unbound ligands can reveal not only modifications that interfere with binding, but also modifications that enhance binding. A ligand can be made with precisely that modification and tested for the enhanced affinity. Thus chemical modification experiments can be a method for exploring additional local contacts with the target molecule, just as walking experiments (see below) are for additional nucleotide level contacts with adjacent domains.

A consensus of primary and secondary structures that enables the chemical or enzymatic synthesis of oligonucleotide ligands whose design is based on that consensus is provided herein via an *in vitro* selection method, preferably a modified *in vitro* selection method as disclosed herein. Because the replication machinery of *in vitro* selection requires that rather limited variation at the subunit level (ribonucleotides, for example), such ligands imperfectly fill the available atomic space of a target molecule's binding surface. However, these ligands can be thought of as high-affinity scaffolds that can be derivatized to make additional contacts with the target molecule. In addition, the consensus contains atomic group descriptors that are pertinent to binding and atomic group descriptors that are coincidental to the pertinent atomic group interactions. Such derivatization does not exclude incorporation of cross-linking agents that will give specifically directly covalent linkages to the target protein. Such derivatization analyses can be performed at but are not limited to the 2' position of the ribose, and thus can also include derivatization at any position in the base or backbone of the nucleotide ligand.

The present invention thus includes nucleic acid ligands wherein certain chemical modifications have been made in order to increase the *in vivo* stability of the ligand or to enhance or mediate the delivery of the ligand. Examples of such modifications include chemical substitutions at the ribose and/or phosphate positions of a given RNA sequence.

A logical extension of this analysis is a situation in which one or a few nucleotides of the polymeric ligand are used as a site for chemical derivative exploration. The rest of the ligand serves to anchor in place this monomer (or monomers) on which a variety of derivatives are tested for non-interference with binding and for enhanced affinity. Such 5 explorations can result in small molecules that mimic the structure of the initial ligand framework, and have significant and specific affinity for the target molecule independent of that nucleic acid framework. Such derivatized subunits, which can have advantages with respect to mass production, therapeutic routes of administration, delivery, clearance 10 or degradation than the initial ligand, can become the therapeutic and can retain very little of the original ligand. Thus, the aptamer ligands of the present invention can allow directed chemical exploration of a defined site on the target molecule known to be important for the target function.

#### Walking Experiments

After a minimal consensus ligand sequence has been determined for a given target, 15 it is possible to add random sequence to the minimal consensus ligand sequence and evolve additional contacts with the target, perhaps to separate but adjacent domains. This procedure has been referred to in the art as "walking". A walking experiment can involve two experiments performed sequentially. A new candidate mixture is produced in which 20 each of the members of the candidate mixture has a fixed nucleic acid region that corresponds to a nucleic acid ligand of interest. Each member of the candidate mixture also contains a randomized region of sequences. According to this method it is possible to identify what are referred to as "extended" nucleic acid ligands, which contain regions that can bind to more than one binding domain of a target.

#### Covariance Analysis

25 In conjunction with empirical methods for determining the three dimensional structure of nucleic acids, computer modeling methods for determining structure of nucleic acid ligands can also be employed. Secondary structure prediction is a useful guide to correct sequence alignment. It is also a highly useful stepping-stone to correct 3D structure prediction, by constraining a number of bases into A-form helical geometry.

Tables of energy parameters for calculating the stability of secondary structures exist. Although early secondary structure prediction programs attempted to simply maximize the number of base-pairs formed by a sequence, most current programs seek to find structures with minimal free energy as calculated by these thermodynamic parameters. There are two problems in this approach that should be borne in mind. First, the thermodynamic rules are inherently inaccurate, typically to 10% or so, and there are many different possible structures lying within 10% of the global energy minimum. Second, the actual secondary structure need not lie at a global energy minimum, depending on the kinetics of folding and synthesis of the sequence. Nonetheless, for short sequences, these caveats are of minor importance because there are so few possible structures that can form.

The brute force predictive method is a dot-plot: make an N by N plot of the sequence against itself, and mark an X everywhere a base pair is possible. Diagonal runs of X's mark the location of possible helices. Exhaustive tree-searching methods can then search for all possible arrangements of compatible (i.e., non-overlapping) helices of length L or more; energy calculations can be done for these structures to rank them as more or less likely. The advantages of this method are that all possible topologies, including pseudoknotted conformations, can be examined, and that a number of suboptimal structures are automatically generated as well. An elegant predictive method, and currently the most used, is the Zuker program. Zuker, 1989, *Science*, 244, 48-52. Originally based on an algorithm developed by Ruth Nussinov, the Zuker program makes a major simplifying assumption that no pseudoknotted conformations will be allowed. This permits the use of a dynamic programming approach that runs in time proportional to only  $N^3$  to  $N^4$ , where N is the length of the sequence. The Zuker program is the only program capable of rigorously dealing with sequences of than a few hundred nucleotides, so it has come to be the most commonly used by biologists. However, the inability of the Zuker program to predict pseudoknotted conformations is a serious consideration. Where pseudoknotted RNA structures are suspected or are recognized by eye, a brute-force method capable of predicting pseudoknotted conformations should be employed.

A central element of comparative sequence analysis is sequence covariations. A covariation is when the identity of one position depends on the identity of another position; for instance, a required Watson-Crick base pair shows strong covariation in that

knowledge of one of the two positions gives absolute knowledge of the identity at the other position. Covariation analysis has been used previously to predict the secondary structure of RNAs for which a number of related sequences sharing a common structure exist, such as tRNA, rRNAs, and group I introns. It is now apparent that covariation analysis can be used to detect tertiary contacts as well. Storno and Gutell, 1992, *Nucleic Acids Research*, 29, 5785-5795 have designed and implemented an algorithm that precisely measures the amount of covariations between two positions in an aligned sequence set. The program is called "MIXY"-Mutual Information at position X and Y. Consider an aligned sequence set. In each column or position, the frequency of occurrence of A, C, G, U, and gaps is calculated. This frequency is called  $f(b_x)$ , the frequency of base b in column x. Considering two columns at once, the frequency that a given base b appears in column x is  $f(b_x)$  and the frequency that a given base b appears in column y is  $f(b_y)$ . If position x and position y do not care about each other's identity—that is, the positions are independent; there is no covariation—the frequency of observing bases  $b_x$  and  $b_y$  at position x and y in any given sequence should be just  $f(b_x b_y) = f(b_x) f(b_y)$ . If there are substantial deviations of the observed frequencies of pairs from their expected frequencies, the positions are said to covary.

The amount of deviation from expectation can be quantified with an information measure  $M(x,y)$ , the mutual information of x and y.

$$M(x,y) = \sum_{b_x b_y} f(b_x b_y) \ln \frac{f(b_x b_y)}{f(b_x) f(b_y)}$$

20

$M(x,y)$  can be described as the number of bits of information one learns about the identity of position y from knowing just the identity of position x. If there is no covariation,  $M(x,y)$  is zero; larger values of  $M(x,y)$  indicate strong covariation. Covariation values can be used to develop three-dimensional structural predictions.

25 In some ways, the problem is similar to that of structure determination by NMR. Unlike crystallography, which in the end yields an actual electron density map, NMR yields a set of interatomic distances. Depending on the number of interatomic distances one can get, there can be one, few, or many 3D structures with which they are consistent. Mathematical techniques had to be developed to transform a matrix of interatomic

distances into a structure in 3D space. The two main techniques in use are distance geometry and restrained molecular dynamics.

Distance geometry is the more formal and purely mathematical technique. The interatomic distances are considered to be coordinates in an N-dimensional space, where 5 N is the number of atoms. In other words, the "position" of an atom is specified by N distances to all the other atoms, instead of the three (x,y,z) coordinates typically considered. Interatomic distances between every atom are recorded in an N-by-N distance matrix. A complete and precise distance matrix is easily transformed into a 3 by N 10 Cartesian coordinates, using matrix algebra operations. The trick of distance geometry as applied to NMR is dealing with incomplete (only some of the interatomic distances are 15 known) and imprecise data (distances are known to a precision of only a few angstroms at best). Much of the time of distance geometry-based structure calculation is thus spent in pre-processing the distance matrix, calculating bounds for the unknown distance values based on the known ones, and narrowing the bounds on the known ones. Usually, 20 multiple structures are extracted from the distance matrix that are consistent with a set of NMR data; if they all overlap nicely, the data were sufficient to determine a unique structure. Unlike NMR structure determination, covariance gives only imprecise distance values; but also only probabilistic rather than absolute knowledge about whether a given 25 distance constraint should be applied.

Restrained molecular dynamics can also be employed, albeit in a more ad hoc manner. Given an empirical force field that attempts to describe the forces that all the atoms feel (van der Waals, covalent bonding lengths and angles, electrostatics), one can simulate a number of femtosecond time steps of a molecule's motion, by assigning every 20 atom at a random velocity (from the Boltzmann distribution at a given temperature) and calculating each atom's motion for a femtosecond using Newtonian dynamical equations; that is "molecular dynamics". In restrained molecular dynamics, one assigns extra ad hoc 25 forces to the atoms when they violate specified distance bounds.

With respect to RNA aptamers, the probabilistic nature of data with restrained 30 molecular dynamics can be addressed. The covariation values can be transformed into artificial restraining forces between certain atoms for certain distance bounds; varying the magnitude of the force according to the magnitude of the covariance. NMR and

covariance analysis generates distance restraints between atoms or positions, which are readily transformed into structures through distance geometry or restrained molecular dynamics. Another source of experimental data which can be utilized to determine the three dimensional structures of nucleic acids is chemical and enzymatic protection 5 experiments, which generate solvent accessibility restraints for individual atoms or positions.

Example 2: Nucleic Acid Molecules for Modulating HIV env Gene Expression

The following examples demonstrate the selection and design of Enzymatic Nucleic Acid (hammerhead, DNAzyme, NCH, Amberzyme, Zinzyme, or G-Cleaver), Antisense, 10 and siRNA molecules and binding/cleavage sites within HIV RNA.

Identification of Potential Target Sites in Human HIV RNA

The sequence of human HIV genes are screened for accessible sites using a computer-folding algorithm. Regions of the RNA that do not form secondary folding structures and contained potential enzymatic nucleic acid molecule and/or antisense 15 binding/cleavage sites are identified. The sequences of these binding/cleavage sites are shown in **Tables III to XI**.

Example 2: Selection of Enzymatic Nucleic Acid Cleavage Sites in Human HIV RNA

Enzymatic nucleic acid molecule target sites are chosen by analyzing sequences of Human HIV (Genbank accession No: NM\_005228) and prioritizing the sites on the basis 20 of folding. Enzymatic nucleic acid molecules are designed that can bind each target and are individually analyzed by computer folding (Christoffersen *et al.*, 1994 *J. Mol. Struc. Theochem.*, 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the enzymatic nucleic acid molecule sequences fold into the appropriate 25 secondary structure. Those enzymatic nucleic acid molecules with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Example 3: Chemical Synthesis and Purification of Ribozymes and Antisense for Efficient Cleavage and/or blocking of HIV RNA

Enzymatic nucleic acid molecules and antisense constructs are designed to anneal to various sites in the RNA message. The binding arms of the enzymatic nucleic acid molecules are complementary to the target site sequences described above, while the antisense constructs are fully complementary to the target site sequences described above. The enzymatic nucleic acid molecules and antisense constructs were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman *et al.*, (1987 *J. Am. Chem. Soc.*, 109, 7845), Scaringe *et al.*, (1990 *Nucleic Acids Res.*, 18, 5433) and Wincott *et al.*, *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were typically >98%.

Enzymatic nucleic acid molecules and antisense constructs are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). Enzymatic nucleic acid molecules and antisense constructs are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Wincott *et al.*, *supra*; the totality of which is hereby incorporated herein by reference) and are resuspended in water. The sequences of the chemically enzymatic nucleic acid molecules used in this study are shown below in **Tables VI to IX**. The sequences of the antisense constructs used in this study are shown in **Table X**. The sequences of the siRNA constructs used in this study are shown in **Table XI**.

Example 4: Enzymatic nucleic acid molecule Cleavage of HIV RNA Target *in vitro*

Enzymatic nucleic acid molecules targeted to the human HIV RNA are designed and synthesized as described above. These enzymatic nucleic acid molecules can be tested for cleavage activity *in vitro*, for example, using the following procedure. The target sequences and the nucleotide location within the HIV RNA are given in **Tables III to IX**.

*Cleavage Reactions:* Full-length or partially full-length, internally-labeled target RNA for enzymatic nucleic acid molecule cleavage assay is prepared by *in vitro* transcription in the presence of [ $\alpha$ -<sup>32</sup>P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification.

5 Alternately, substrates are 5'-<sup>32</sup>P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified enzymatic nucleic acid molecule in enzymatic nucleic acid molecule cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl<sub>2</sub>) and the cleavage reaction was initiated by adding the 2X enzymatic nucleic acid molecule mix to an equal volume of substrate RNA (maximum of 10 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM enzymatic nucleic acid molecule, *i.e.*, enzymatic nucleic acid molecule excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is 15 heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by enzymatic nucleic acid molecule cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager® quantitation of bands representing the intact substrate and the cleavage products.

20 Indications

Particular degenerative and disease states that can be associated with HIV expression modulation include but are not limited to acquired immunodeficiency disease (AIDS) and related diseases and conditions, including but not limited to Kaposi's sarcoma, lymphoma, cervical cancer, squamous cell carcinoma, cardiac myopathy, 25 rheumatic diseases, and opportunistic infection, for example *Pneumocystis carinii*, *Cytomegalovirus*, *Herpes simplex*, *Mycobacteria*, *Cryptococcus*, *Toxoplasma*, *Progressive multifocal leucoencephalopathy* (*Papovavirus*), *Mycobacteria*, *Aspergillus*, *Cryptococcus*, *Candida*, *Cryptosporidium*, *Isospora belli*, *Microsporidia* and any other 30 diseases or conditions that are related to or will respond to the levels of HIV in a cell or tissue, alone or in combination with other therapies

The present body of knowledge in HIV research indicates the need for methods to assay HIV activity and for compounds that can regulate HIV expression for research, diagnostic, and therapeutic use.

The use of antiviral compounds, monoclonal antibodies, chemotherapy, radiation therapy, analgesics, and/or anti-inflammatory compounds, are all non-limiting examples of methods that can be combined with or used in conjunction with the nucleic acid molecules (e.g. aptamers, siRNA, antisense, and enzymatic nucleic acid molecules) of the instant invention. Examples of antiviral compounds that can be used in conjunction with the nucleic acid molecules of the invention include but are not limited to AZT (also known as zidovudine or ZDV), ddC (zalcitabine), ddI (dideoxyinosine), d4T (stavudine), and 3TC (lamivudine) Ribavirin, delvaridine (Rescriptor), nevirapine (Viramune), efavirenz (Sustiva), ritonavir (Norvir), saquinavir (Invirase), indinavir (Crixivan), amprenavir (Agenerase), nelfinavir (Viracept), and/or lopinavir (Kaletra). Common chemotherapies that can be combined with nucleic acid molecules of the instant invention include various combinations of cytotoxic drugs to kill cancer cells. These drugs include but are not limited to paclitaxel (Taxol), docetaxel, cisplatin, methotrexate, cyclophosphamide, doxorubicin, fluorouracil carboplatin, edatrexate, gemcitabine, vinorelbine etc. Those skilled in the art will recognize that other drug compounds and therapies can be similarly be readily combined with the nucleic acid molecules of the instant invention are hence within the scope of the instant invention.

#### Diagnostic uses

The aptamers of the invention can be used to detect the presence or absence of the target substances to which they specifically bind, such as gp41 or gp120. Such diagnostic tests are conducted by contacting a sample with the aptamer to obtain a complex that is then detected by conventional techniques known in the art. For example, the aptamers can be labeled using radioactive, fluorescent, or chromogenic labels. Interaction of labeled aptamer with the target can result in the detection of the target molecule via an ELISA type assay or sandwich assay, or by other means known in the art. Alternately, the aptamers of the invention can be used to separate or isolate molecules that specifically bind to the aptamer. For example, by coupling the aptamers to a solid support, target

molecules which bind to the aptamers can be recovered via affinity chromatography or analyzed by standard means known in the art.

The enzymatic nucleic acid molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence 5 of HIV RNA in a cell. The close relationship between enzymatic nucleic acid molecule activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple enzymatic nucleic acid molecules described in this invention, one can map nucleotide changes which are important to RNA structure and 10 function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with enzymatic nucleic acid molecules can be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets can be defined as important mediators of the disease. These 15 experiments can lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple enzymatic nucleic acid molecules targeted to different genes, enzymatic nucleic acid molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations of enzymatic nucleic acid molecules and/or other chemical or biological molecules). Other *in vitro* uses of enzymatic nucleic acid molecules of this invention are well known in the art, and include 20 detection of the presence of mRNAs associated with HIV-related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with an enzymatic nucleic acid molecule using standard methodology.

In a specific example, enzymatic nucleic acid molecules which cleave only wild-type or mutant forms of the target RNA are used for the assay. The first enzymatic 25 nucleic acid molecule is used to identify wild-type RNA present in the sample and the second enzymatic nucleic acid molecule is used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both enzymatic nucleic acid molecules to demonstrate the relative enzymatic nucleic acid molecule efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample 30 population. Thus each analysis requires two enzymatic nucleic acid molecules, two

substrates and one unknown sample which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, HIV) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively. The use of enzymatic nucleic acid molecules in diagnostic applications contemplated by the instant invention is more fully described in George *et al.*, US Patent Nos. 5,834,186 and 5,741,679, Shih *et al.*, US Patent No. 5,589,332, Nathan *et al.*, US Patent No 5,871,914, Nathan and Ellington, International PCT publication No. WO 00/24931, Breaker *et al.*, International PCT Publication Nos. WO 00/26226 and 98/27104, and Sullenger *et al.*, International PCT publication No. WO 99/29842.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the 5 scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

10 In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

**Table I: HIV env sequences (Subtype. Country. isolate year. isolate name)**

HIV env sequences
A.BY.97.97BL006
A.GB.-.MA246
A.GB.-.MC108
A.KE.90.K89
A.RW.-.PVPI
A.RW.-.SF1703
A.SE.94.SE7535
A.SE.95.SE8538
A.SE.95.SE8891
A.SE.95.UGSE8131
A.UA.97.ukr970063
A.UG.90.UG273A
A.UG.90.UG275A
A1.KE.93.Q23-17
A1.SE.94.SE7253
A1.UG.85.U455
A1.UG.92.92UG037
A2.CD.-.97CDKS10
A2.CD.-.97CDKTB48
A2.CY.94.94CY017.41
A2C.ZM.89.ZAM174
A2C.ZM.89.ZAM716-3
A2C.ZM.90.ZAM18B
A2D.KR.97.97KR004
A2G.CD.-.97CDKP58
AC.BE.-.VI313
AC.IN.95.95IN21301
AC.RW.92.92RW009
AC.SE.96.SE9488
ACD.SE.95.SE8603
ACG.BE.-.VI1035
AD.KE.90.K124A2
AD.SE.93.SE6954
AD.SE.95.SE7108
AD.UG.-.C6080-10
AD.UG.92.2UG035-22
ADHK.NO.97.97NOGIL3
ADK.CD.85.MAL
AG.BE.-.VI1197
AG.BE.-.VI5251
AG.CD.89.VI191A2
AG.NG.92.92NG003
AGHU.GA.-.VI354
AGJ.BW.98.BW2117
AGU.CD.76.Z321
AU.NG.94.NG3678
AU.NG.95.NG1935
AU.SE.93.SE6594
B.AU.-.VH
B.AU.86.MBC200

B.AU.87.MBC925
B.AU.93.MBC18
B.AU.95.MBCC54
B.AU.96.MBCC98
B.AU.96.MBCD36
B.BE.-.SIMI84
B.CA.-.CA5
B.CN.-.RL42
B.DE.86.D31
B.DE.86.HAN
B.ES.89.89SP061
B.FR.-.PHI120
B.FR.-.PHI133
B.FR.-.PHI146
B.FR.-.PHI153
B.FR.-.PHI159
B.FR.-.PIH155
B.FR.-.PIH160
B.FR.-.PIH309
B.FR.-.PIH373
B.FR.-.PIH374
B.FR.83.HXB2
B.GA.-.OYI,
B.GB.-.AC-46
B.GB.-.CAM1
B.GB.-.GB8.C1
B.GB.-.HIM286332
B.GB.-.HIM286336
B.GB.-.HIM286337
B.GB.-.JB
B.GB.-.M23470
B.GB.-.M24244C3
B.GB.-.M26864
B.GB.-.M30156
B.GB.-.M737677
B.GB.-.M737685
B.GB.-.MB314
B.GB.-.PE052C1
B.GB.-.PE104C38
B.GB.-.PE131C3
B.GB.-.WB
B.GB.59.MANC
B.JP.-.ETR
B.JP.86.JH32
B.KR.-.WK
B.NL.-.68A
B.NL.-.ENVVA
B.NL.-.ENVVF
B.NL.-.ENVVG
B.NL.86.3202A21
B.NL.86.H0320-2A12
B.TH.93.93TH067
B.TT.-.QZ4589
B.TW.-.TWCYS

B.UA.-.UKR1216
B.UNK.-.NL43E9
B.US.-.546BMB4
B.US.-.ADA
B.US.-.BORI
B.US.-.BRVA
B.US.-.C26-12.1BH
B.US.-.DH123
B.US.-.M02-3.SW
B.US.-.NC7
B.US.-.P896
B.US.-.SF128A
B.US.-.US1
B.US.-.US2
B.US.-.US3
B.US.-.US4
B.US.-.WMJ22
B.US.83.RF
B.US.83.SF2
B.US.84.CDC452
B.US.84.MNCG
B.US.84.NY5CG
B.US.84.SC
B.US.84.SC141
B.US.84.SC14C
B.US.85.85WCIPR54
B.US.85.AL1
B.US.85.SFMHS11
B.US.85.SFMHS21
B.US.85.SFMHS3
B.US.86.JRCSF
B.US.86.JRFL
B.US.86.SFMHS1
B.US.86.SFMHS16
B.US.86.SFMHS17
B.US.86.SFMHS18
B.US.86.SFMHS2
B.US.86.SFMHS4
B.US.86.SFMHS8
B.US.86.YU2
B.US.87.BC
B.US.87.SFMHS5
B.US.87.SFMHS7
B.US.87.SFMHS9
B.US.88.SFMHS19
B.US.88.SFMHS6
B.US.88.WR27
B.US.89.R2
B.US.89.SFMHS20
B.US.90.WEAU160
B.US.92.92US657.1
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C.BI.91.BU910213
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C.BI.91.BU910423
C.BI.91.BU910518
C.BI.91.BU910611
C.BI.91.BU910717
C.BI.91.BU910812
C.BR.92.92BR025
C.BW.96.96BW01B03
C.BW.96.96BW0402
C.BW.96.96BW0502
C.BW.96.96BW11B01
C.BW.96.96BW1210
C.BW.96.96BW15B03
C.BW.96.96BW16B01
C.BW.96.96BW17B05
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C.ET.86.ETH2220
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C.IN.93.93IN101
C.IN.93.93IN904
C.IN.93.93IN905
C.IN.93.93IN999
C.IN.94.94IN11246
C.IN.95.95IN21068
C.SN.90.SE364A
C.SO.89.SO145A
C.UG.90.UG268A2
CD.BI.91.BU910905
CPZ.CD.-.CPZANT
CPZ.CM.-.CAM3
CPZ.CM.98.CAM5
CPZ.GA.-.CPZGAB
CPZ.US.85.CPZUS
CRF01 AE.CF.90.90CF1
CRF01 AE.CF.90.90CF4
CRF01 AE.CM.-.CA10
CRF01 AE.TH.90.CM240
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CRF01 AE.TH.92.TH921
CRF01 AE.TH.93.93TH0
CRF01 AE.TH.93.93TH2
CRF01 AE.TH.93.KH03
CRF01 AE.TH.93.KH08
CRF01 AE.TH.94.A0102
CRF01 AE.TH.94.E1142
CRF01 AE.TH.95.95TNI
CRF01 AE.TH.95.NI114
CRF01 AE.TH.95.NI115
CRF01 AE.TH.95.TH022
CRF01 AE.TH.96.NI115
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CRF02 AG.DJ.91.DJ258

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CRF02_AG.FR.91.DJ264
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CRF02_AG.NG.95.NG192
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CRF02_AG.SN.98.MP121
CRF03_AB.RU.-.KAL68.
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CRF04_cpx.GR.91.97PV
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CRF05_DF.BE.93.VI961
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CRF06_cpx.ML.95.95ML
CRF06_cpx.NG.94.NG36
CRF06_cpx.SN.97.97SE
CRF10_CD.TZ.96.96TZB
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CRF11_cpx.NG.94.NG36
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D.CD.83.ELI
D.CD.83.NDK
D.CD.84.84ZR085
D.CD.85.Z2Z6
D.CI.-.CI13
D.SN.90.SE365A2
D.TZ.87.87TZ4622
D.UG.-.C971-412
D.UG.-.WHO15-474
D.UG.90.UG266A2
D.UG.90.UG269A
D.UG.90.UG274A2
D.UG.92.92UG024-D
D.UG.94.94UG1141
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F1.BR.89.BZ126
F1.BR.93.93BR020.1
F1.FI.93.FIN9363
F1.FR.96.MP411
F2.CM.-.CA20
F2.CM.-.HIM277819
F2.CM.95.MP255
F2.CM.95.MP257
F2KU.BE.94.VI1126
G.BE.96.DRCBL
G.FI.93.HH8793-12.1

G.GA.-.LBV217
G.NG.92.92NG083
G.NG.95.NG1928
G.NG.95.NG1929
G.NG.95.NG1937
G.NG.95.NG1939
G.SE.93.SE6165
GH.GA.90.VI525
H.BE.93.VI991
H.BE.93.VI997
H.CF.90.90CF056
J.SE.93.SE7887
J.SE.94.SE7022
K.CD.97.EQTB11C
K.CM.96.MP535
MO.CM.97.97CAMP645MO
N.CM.-.YBF106
N.CM.95.YBF30
O.CM.-.ANT70
O.CM.-.CM4974
O.CM.91.MVP5180
O.CM.93.HIV1CA9EN
O.GA.92.VI686
O.GQ.-.193HA
O.GQ.-.276HA
O.GQ.-.341HA
O.SN.99.SEMP1299
O.SN.99.SEMP1300
U.CD.83.83CD003

**Table II:****A. 2.5 µmol Synthesis Cycle ABI 394 Instrument**

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 µL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 µL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 µL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

**B. 0.2 µmol Synthesis Cycle ABI 394 Instrument**

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 µL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 µL	45 sec	233 min	465 sec
Acetic Anhydride	655	124 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 µL	5 sec	5 sec	5 sec
TCA	700	732 µL	10 sec	10 sec	10 sec
Iodine	20.6	244 µL	15 sec	15 sec	15 sec
Beaucage	7.7	232 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2  $\mu$ mol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O- methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O- methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 $\mu$ L	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 $\mu$ L	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 $\mu$ L	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 $\mu$ L	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 $\mu$ L	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 $\mu$ L	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 $\mu$ L	NA	NA	NA

- Wait time does not include contact time during delivery.

**Table III: HIV env target sequences**

Sequence	Seq ID
AAAAAAUAACAUGGUA	1
AAAAAAUUAUCAUAAU	2
AAAAGAAUGAACAAAG	3
AAAAUAACAUGGUAG	4
AAACUGCUCUUCAA	5
AAAGAAUGAACAAAGA	6
AAAGAGAAGAGUGGU	7
AAAGCCAUGUGUAAA	8
AAAGCCUAAAGCCAU	9
AAAUAACAUGGUAGA	10
AAAUAUAAAGUAGUA	11
AACAUGACCUGGAUG	12
AACAUGUGGAAAAAU	13
AACGCUGACGGUACA	14
AACUCACAGUCUGGG	15
AAGAAGAAGGUGGGAG	16
AAGAGAAGAGUGGUG	17
AAGCAAUGUAUGGCC	18
AAGCACUAUGGGCGC	19
AAGCCUAAAGCCAUG	20
AAGUGAAUUUAUAAA	21
AAUAAACGCUGACGGU	22
AAUAGAGUUAGGCAG	23
AAUAUUCAUAAUGAU	24
AAUCAGUUUAUGGGA	25
AAUGAUAGUAGGAGG	26
AAUGGCAGUCUAGCA	27
AAUGUACACAUUGGAA	28
AAUGUAUGCCCCUCC	29
AAUGUCAGCACAGUA	30
AAUUUAUAAAUAUAAA	31
AAUUCCCAUACAUUA	32
AAUUGGAGAAGUGAA	33
AAUUJUGCUGAGGGCU	34
ACAAUGUACACAUGG	35
ACAAUUAUUGUCUGG	36
ACAAUUGGAGAAGUG	37
ACACAUGCCUGUGUA	38
ACAGACCCCAACCCA	39
ACAGGCCAGACAAUU	40
ACAGUACAAUGUACA	41
ACAGUCUAAAUGGG	42
ACAUGCCUGUGUACC	43
ACAUGUGGAAAAUA	44
ACCACCGCUUGAGAG	45
ACCCACAGACCCCAA	46
ACCCCAACCCACAAG	47
ACCUGGGAGGAGGAGA	48
ACCUGUGUGGAAAGA	49
ACGCUGACGGUACAG	50
ACGGUACAGGCCAGA	51

ACUCACAGUCUGGGG	52
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AGAAGAAGGUGGAGA	54
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AGACAAUUAUUGUCU	56
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AGCACUAUGGGCGCA	64
AGCAGCAGGAAGCAC	65
AGCAGGAAGCACUAU	66
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AGGAAGCACUAUGGG	70
AGGAGGAGAU AUGAG	71
AGGAGUAGCACCCAC	72
AGGAUCAACAGCUCC	73
AGGCAAGAGGUCCUGG	74
AGGCAGGGAUACUCA	75
AGGGACAAUUGGGAGA	76
AGUACAAUGUACACA	77
AGUCUAAUUAUGGGGU	78
AGUGAAUUAUUAUAAA	79
AGUUAGGCAGGGAU	80
AGUUGGAGUAAUAAA	81
AGUUUAUGGGAUCAA	82
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AUAAUAAUCAGUUUAU	91
AUAUGAGGGACAAUU	92
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AUCAACAGCUCCUAG	95
AUCAGAUGCUAAAGC	96
AUCAGUUUAUGGGAU	97
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AUGGGGUACCUGUGU	102
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AUGUCAGCACAGUAC	105

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AUUCAUAAUGAUAGU	110
AUUCCAUACAUUAU	111
AUUGGAGAAGUGAAU	112
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AUUUUGUGCAUCAGA	116
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CAAAGCCUAAAGCCA	118
CAACUCACAGUCUGG	119
CAAUAACGCUGACGG	120
CAAUGUACACAUAGGA	121
CAAUGUAUGCCCCUC	122
CAAUCCCAUACAUU	123
CAAUJUGGAGAAGUGA	124
CAAUUUGCUGAGGGC	125
CACAGACCCCAACCC	126
CACAGUCUAAUUAUGG	127
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CACAUGCUGUGUAC	129
CACUAUGGGCGCAGC	130
CAGACAAUUAUUGUC	131
CAGACCCCAACCCAC	132
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CAGCACAGUACAAUG	135
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CAGCUCCAGGCAAGA	137
CAGGAAGCACUAUGG	138
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CAGGCCAGACAAUUA	140
CAGUACAAUGUACAC	141
CAGUCUAAUUAUGGGG	142
CAGUUUAUGGGAUCA	143
CAUAUAGAUAGUAGG	144
CAUACAUUAUUGUGC	145
CAUCAGAUGCUALAG	146
CAUGCUGUGUACCC	147
CAUGUGGAAAAUAUA	148
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CCACAGACCCCAACC	150
CCACCGCUUGAGAGA	151
CCAGACAAUUAUUGU	152
CCAGGCAAGAGUCCU	153
CCAUACAUUAUUGUG	154
CCCAACCCACAAGAA	155
CCCACAGACCCCAAC	156
CCCAUACAUUAUUGU	157
CCCCAACCCACAAGA	158
CCGUUGAGAGACUU	159

CCUAAAAGCCAUGUGU	160
CCUCUUCAGCUACCA	161
CCUGGAGGAGGAGAU	162
CCUGGCUGUGGAAAG	163
CCUGUGCCUCUUCAG	164
CCUGUGUACCCACAG	165
CCUUGGGUUCUUGGG	166
CCUUUGAGCCAUAUUC	167
CGCUGACGGUACAGG	168
CGGUACAGGCCAGAC	169
CUAAAGCCAUGUGUA	170
CUAAAGGAUCAACAG	171
CUAGUUGGAGUAAUA	172
CUAUUAUGGGGUACC	173
CUAUUUJUGUGCAUCA	174
CUCACAGUCUGGGGC	175
CUCCAGGCAAGAGUC	176
CUCUGGAAAACUCAU	177
CUCUUCAGCUACCAC	178
CUGACGGUACAGGCC	179
CUGGAGGAGGAGAUA	180
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CUGUGGUUAUAAA	185
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GAAAAGAAUGAACAA	193
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GAGCCUGUGCCUCUU	208
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GAGUUAGGCAGGGAU	211
GAUAGUAGGAGGCUU	212
GAUAAAUCAGUUUA	213

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GAUGCUAAAGCAUAU	215
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GCAAGAGUCCUGGCU	217
GCAAUGUAUGCCCCU	218
GCACUAUGGGCGCAG	219
GCAGCAGGAAGCACU	220
GCAGGAAGCACUAUG	221
GCAGGGAUACUCACC	222
GCAUCAGAUGCUAAA	223
GCCAGACAAUUAUUG	224
GCCUAAAGCCAUGUG	225
GCCUCUUCAGCUACC	226
GCCUGUGCCUCUCA	227
GCCUGUGUACCCACA	228
GUCCAGGGCAAGAGU	229
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UACCCACAGACCCCA	297
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UCAACAGCUCCUAGG	317
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UCAGACCUGGAGGAG	321

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UCAGCACAGUACAAU	323
UCAGUUUAUGGGAUC	324
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UCCUGGCUGUGGGAAA	328
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UCUAAUUAUGGGGUAC	330
UCUGGUAUAGUGCAA	331
UCUUCAGCUACCACC	332
UCUUGGGAGCAGCAG	333
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UGUAUGCCCCUCCCA	360
UGUCAGCACAGUACA	361
UGUCUGGUAUAGUGC	362
UGUGCAUCAGAUGCU	363
UGUGCCUCUUCAGCU	364
UGUGGAAAAUAACA	365
UGUGGAAAGAUACCU	366
UGUGGAACUUCUGGG	367
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UGUUCCUUGGGUUCU	370
UGUUGCAACUCACAG	371
UUAACAUGUGGAAA	372
UUAAGAAUAGUUUUU	373
UUAGGCAGGGAUACU	374
UUUAUAAAUAUAAA	375

UUAUGGGGUACCUUGU	376
UUAUUGUCUGGUUAUA	377
UUCAGACCUGGAGGA	378
UUCAUAAUGAUAGUA	379
UUCCCAUACAUUAUU	380
UUCCUUGGGUUCUUG	381
UUCUUGGGAGCAGCA	382
UUGCAACUCACAGUC	383
UUGGAGAAAGUGAAUU	384
UUGGAGUAAUAAAUC	385
UUGGGAGCAGCAGGA	386
UUGGGUUCUUGGGAG	387
UUGUCUGGUAUAGUG	388
UUGUGCAUCAGAUGC	389
UUUAACAUGUGGAAA	390
UUUAUGGGAUCAAAG	391
UUUGCUGAGGGCUAU	392
UUUGUGCAUCAGAUG	393
UUUUACAUGUGGAA	394
UUUUGUGCAUCAGAU	395

Table IV: HIV env Target and Hammerhead Sequence

Substrate	Seq ID	Hammerhead Ribozyme	Seq ID
AAAAAU A CAUGGUA	1	UACCAUG CUGAUGAGGCCGUUAGGCCGAA UAUUUUU	505
AAAAAU A UCAUAAU	2	AUUAUGA CUGAUGAGGCCGUUAGGCCGAA UAUUUUU	506
AAAUUA A AGUAGUA	11	UACUACU CUGAUGAGGCCGUUAGGCCGAA UAUAUUU	507
AAGCCUA A AGCCAUG	20	CAUGGCU CUGAUGAGGCCGUUAGGCCGAA UAGGCUU	508
AAUAUUC A UAAUGAU	24	AUCAUUA CUGAUGAGGCCGUUAGGCCGAA GAAUAUU	509
AAUGAUA G UAGGAGG	26	CCUCCUA CUGAUGAGGCCGUUAGGCCGAA UAUCAUU	510
AAUUUA U AAAUUA	31	UAUAUUU CUGAUGAGGCCGUUAGGCCGAA UAUAAUU	511
ACAAUUA U UGUCUGG	36	CCAGACA CUGAUGAGGCCGUUAGGCCGAA UAAUUGU	512
AGAGUUA G GCAGGGA	61	UCCCUGC CUGAUGAGGCCGUUAGGCCGAA UAACUCU	513
AGCACUA U GGGCGCA	64	UGCGCCC CUGAUGAGGCCGUUAGGCCGAA UAGUGCU	514
AGGAGUA G CACCCAC	72	GUGGGUG CUGAUGAGGCCGUUAGGCCGAA UACUCCU	515
AUAAAUA U AAAGUAG	84	CUACUUU CUGAUGAGGCCGUUAGGCCGAA UAUUUAU	516
AUCAGUU U AUGGGAU	97	AUCCCAU CUGAUGAGGCCGUUAGGCCGAA AACUGAU	517
AUUCAUA A UGAUAGU	110	ACUAUCA CUGAUGAGGCCGUUAGGCCGAA UAUGAAU	518
CAAUGUA C ACAUGGA	121	UCCAUGU CUGAUGAGGCCGUUAGGCCGAA UACAUUG	519
CAAUGUA U GCCCCUC	122	GAGGGGC CUGAUGAGGCCGUUAGGCCGAA UACAUUG	520
CACAGUC U AUUAUGG	127	CCAUAUU CUGAUGAGGCCGUUAGGCCGAA GACUGUG	521
CACAGUC U GGGGCAU	128	AUGCCCC CUGAUGAGGCCGUUAGGCCGAA GACUGUG	522
CAGUCUA U UAUUGGG	142	CCCCAU A CUGAUGAGGCCGUUAGGCCGAA UAGACUG	523
CAGUUUA U GGGAUCA	143	UGAUCCC CUGAUGAGGCCGUUAGGCCGAA UAAACUG	524
CCAAUUC C CAUACAU	149	AUGUAUG CUGAUGAGGCCGUUAGGCCGAA GAAUUGG	525
CCUCUUC A GCUACCA	161	UGGUAGC CUGAUGAGGCCGUUAGGCCGAA GAAGAGG	526
CUAUUU G UGCAUCA	174	UGAUGCA CUGAUGAGGCCGUUAGGCCGAA AAAAUAG	527
CUGUGUA C CCACAGA	186	UCUGUGG CUGAUGAGGCCGUUAGGCCGAA UACACAG	528
GACAAUU A UUGUCUG	199	CAGACAA CUGAUGAGGCCGUUAGGCCGAA AAUUGUC	529
GACAAUU G GAGAAGU	200	ACUUCUC CUGAUGAGGCCGUUAGGCCGAA AAUUGUC	530
GACGGUA C AGGCCAG	203	CUGGCCU CUGAUGAGGCCGUUAGGCCGAA UACCGUC	531
GAUAGUA G GAGGCUU	212	AAGCCUC CUGAUGAGGCCGUUAGGCCGAA UACUAUC	532
GAUGCUA A AGCAUAU	215	AUAUGCU CUGAUGAGGCCGUUAGGCCGAA UAGCAUC	533
GCAACUC A CAGUCUG	216	CAGACUG CUGAUGAGGCCGUUAGGCCGAA GAGUUGC	534
GCCUCUU C AGCUACC	226	GGUAGCU CUGAUGAGGCCGUUAGGCCGAA AAGAGGC	535
GGCAGUC U AGCAGAA	244	UUCUGCU CUGAUGAGGCCGUUAGGCCGAA GACUGCC	536
GGUUCUU G GGAGCAG	259	CUGCUCC CUGAUGAGGCCGUUAGGCCGAA AAGAACC	537
GUAAUA A AAAUAUU	266	AAUAUUU CUGAUGAGGCCGUUAGGCCGAA UAUAUAC	538
GUCAAUA A CGCUGAC	268	GUCAGCG CUGAUGAGGCCGUUAGGCCGAA UAUUGAC	539
GUCUAUU A UGGGUUA	271	UACCCCA CUGAUGAGGCCGUUAGGCCGAA AAUAGAC	540
GUGAAUU A UAUAAA	273	AUUUAUA CUGAUGAGGCCGUUAGGCCGAA AAUUCAC	541
GUGCAUC A GAUGCUA	274	UAGCAUC CUGAUGAGGCCGUUAGGCCGAA GAUGCAC	542
GUGCCUC U UCAGCUA	275	UAGCUGA CUGAUGAGGCCGUUAGGCCGAA GAGGCAC	543
GUGGGUC A CAGUCUA	279	UAGACUG CUGAUGAGGCCGUUAGGCCGAA GACCCAC	544
GUUCCUU G GGUUCUU	283	AAGAAC CUGAUGAGGCCGUUAGGCCGAA AAGGAAC	545
UAGAGUU A GGCAGGG	300	CCCUGCC CUGAUGAGGCCGUUAGGCCGAA AACUCUA	546
UAUAAUC A GUUUAUG	306	CAUAAAC CUGAUGAGGCCGUUAGGCCGAA GAUUAUA	547
UAUUGUC U GGUAUAG	315	CUAUACC CUGAUGAGGCCGUUAGGCCGAA GACAAUA	548
UCAGUUU A UGGGAUC	324	GAUCCA CUGAUGAGGCCGUUAGGCCGAA AAACUGA	549
UCCCAUA C AUUAUUG	327	CAAUAAU CUGAUGAGGCCGUUAGGCCGAA UAUGGGA	550
UCUAAUA U GGGUAC	330	GUACCCC CUGAUGAGGCCGUUAGGCCGAA UAAUAGA	551
UCUGGUA U AGUGCAA	331	UUGCACU CUGAUGAGGCCGUUAGGCCGAA UACCAGA	552
UGAAUUA U AUAAAUA	334	UAUUUAU CUGAUGAGGCCGUUAGGCCGAA UAAUUCU	553
UGGAGUA A UAAAUCU	348	AGAUUUA CUGAUGAGGCCGUUAGGCCGAA UACUCCA	554

UGGGGUUA C CUGUGUG	353	CACACAG CUGAUGAGGCCGUUAGGCCGAA UACCCCA	555
UGGGUUC U UGGGAGC	355	GCUCCCCA CUGAUGAGGCCGUUAGGCCGAA GAACCCA	556
UGGUUAUA G UGCAACA	356	UGUUGCA CUGAUGAGGCCGUUAGGCCGAA UAUACCA	557
UGGUUAUA U AAAAAUA	357	UAUUUUU CUGAUGAGGCCGUUAGGCCGAA UAUACCA	558
UGUGGUUA U AUAAAAAA	368	UUUUUAU CUGAUGAGGCCGUUAGGCCGAA UACCACA	559
UUAUUAUA A AUAUAAA	375	UUUUAU CUGAUGAGGCCGUUAGGCCGAA UAUUAUA	560
UUGGGUU C UUGGGAG	387	CUCCCCAA CUGAUGAGGCCGUUAGGCCGAA AACCCAA	561

Table V: HIV env Target and Inozyme Sequence

Substrate	Seq ID	Inozyme	Seq ID
AAAGCCA U GUGUAAA	8	UUUACAC CUGAUGAGGCCGUUAGGCCGAA IGGCUUU	562
AAAGCCU A AAGCCAU	9	AUGGCUU CUGAUGAGGCCGUUAGGCCGAA IGGCUUU	563
AAGCACU A UGGCGC	19	GCGCCCA CUGAUGAGGCCGUUAGGCCGAA IGUGCUU	564
AAUGGCA G UCUAGCA	27	UGCUAGA CUGAUGAGGCCGUUAGGCCGAA IGCCAUU	565
AAUGUCA G CACAGUA	30	UACUGUG CUGAUGAGGCCGUUAGGCCGAA IGACAUU	566
AAUUCCC A UACAUUA	32	UAAUGUA CUGAUGAGGCCGUUAGGCCGAA IGGAAUU	567
ACAGACC C CAACCCA	39	UGGGUUG CUGAUGAGGCCGUUAGGCCGAA IGUCUGU	568
ACAGGCC A GACAAUU	40	AAUUGUC CUGAUGAGGCCGUUAGGCCGAA IGCCUGU	569
ACAGUCU A UUAUGGG	42	CCCAUAA CUGAUGAGGCCGUUAGGCCGAA IGACUGU	570
ACAUGCC U GUGUACC	43	GGUACAC CUGAUGAGGCCGUUAGGCCGAA IGCAUGU	571
ACCCACA G ACCCCAA	46	UJGGGGU CUGAUGAGGCCGUUAGGCCGAA IGUGGGU	572
ACUCACA G UCUGGGG	52	CCCCAGA CUGAUGAGGCCGUUAGGCCGAA IGUGAGU	573
AGACCCC A ACCCACA	57	UGUGGGU CUGAUGAGGCCGUUAGGCCGAA IGGGUCU	574
AGAUGCU A AAGCAUA	62	UAUGCUU CUGAUGAGGCCGUUAGGCCGAA IGCAUCU	575
AGCAGCA G GAAGCAC	65	GUGCUUC CUGAUGAGGCCGUUAGGCCGAA IGGUGCU	576
AGCUCCA G GCAAGAG	69	CUCUUGC CUGAUGAGGCCGUUAGGCCGAA IGGAGCU	577
AGGAUCA A CAGCUCC	73	GGAGCUG CUGAUGAGGCCGUUAGGCCGAA IGAUCCU	578
AGGGACA A UJGGAGA	76	UCUCCAA CUGAUGAGGCCGUUAGGCCGAA IGUCCU	579
AUAAUCA G UUUUAUGG	86	CCAUAAA CUGAUGAGGCCGUUAGGCCGAA IGAUUAU	580
AUAAUCA U AAUGAUA	93	UAUCAUU CUGAUGAGGCCGUUAGGCCGAA IGAUUAU	581
AUCAACA G CUCCUAG	95	CUAGGAG CUGAUGAGGCCGUUAGGCCGAA IGUUGAU	582
AUGUACA C AUGGAAU	103	AUUCCAU CUGAUGAGGCCGUUAGGCCGAA IGUACAU	583
AUUAACA A GAGAUGG	107	CCAUCUC CUGAUGAGGCCGUUAGGCCGAA IGUUAAU	584
AUUCCCA U ACAUUAU	111	AUAAUGU CUGAUGAGGCCGUUAGGCCGAA IGGAAU	585
AUUGUCU G GUAUAGU	113	ACUAAUC CUGAUGAGGCCGUUAGGCCGAA IGAACAU	586
AUUJGCU G AGGGCUA	114	UAGCCCU CUGAUGAGGCCGUUAGGCCGAA IGCIAAU	587
CAAAGCC U AAAGCCA	118	UGGCUUU CUGAUGAGGCCGUUAGGCCGAA IGCUUJG	588
CAACUCA C AGUCUGG	119	CCAGACU CUGAUGAGGCCGUUAGGCCGAA IGGAGUJG	589
CAAUUCC C AUACAUU	123	AAUGUAU CUGAUGAGGCCGUUAGGCCGAA IGAUJUG	590
CAGACCC C AACCCAC	132	GUGGGUU CUGAUGAGGCCGUUAGGCCGAA IGGUCUG	591
CAGACCU G GAGGAGG	133	CCUCUC CUGAUGAGGCCGUUAGGCCGAA IGGUCUG	592
CAGCACA G UACAAUG	135	CAUUGUA CUGAUGAGGCCGUUAGGCCGAA IGUGCUG	593
CAGCUCC A GGCAAGA	137	UCUJGCG CUGAUGAGGCCGUUAGGCCGAA IGGAGCUG	594
CAGGCCA G ACAAUUA	140	UAAUUGU CUGAUGAGGCCGUUAGGCCGAA IGGCCUG	595
CAGUACA A UGUACAC	141	GUGUACA CUGAUGAGGCCGUUAGGCCGAA IGUACUG	596
CAUGCCU G UGUACCC	147	GGGUACA CUGAUGAGGCCGUUAGGCCGAA IGGCAUG	597
CCAGACA A UUAAJGU	152	ACAAUAA CUGAUGAGGCCGUUAGGCCGAA IGGCUGG	598
CCAGGCA A GAGUCCU	153	AGGACUC CUGAUGAGGCCGUUAGGCCGAA IGGCUUGG	599
CCAUACA U UAUUGUG	154	CACAAUA CUGAUGAGGCCGUUAGGCCGAA IGUAUJG	600
CCCAACC C ACAAGAA	155	UUCUJGU CUGAUGAGGCCGUUAGGCCGAA IGUUGGG	601
CCUGGGU G UGGAAAG	163	CUUJCCA CUGAUGAGGCCGUUAGGCCGAA IGGCAGG	602
CGGUACA G GCCAGAC	169	GUCUGGC CUGAUGAGGCCGUUAGGCCGAA IGUACCG	603
CUCUUCA G CUACCAC	178	GUGGUAG CUGAUGAGGCCGUUAGGCCGAA IGAAGAG	604
CUGUGCC U CUUCAGC	183	GCUGAAG CUGAUGAGGCCGUUAGGCCGAA IGGCACAG	605
GACCCCA A CCCACAA	201	UUGUGGG CUGAUGAGGCCGUUAGGCCGAA IGGGGUC	606
GGAAGCA C UAUGGGC	235	GCCCAUA CUGAUGAGGCCGUUAGGCCGAA IGGCUCC	607
GGAGCCU G UGCCUCU	239	AGAGGCA CUGAUGAGGCCGUUAGGCCGAA IGGCUCC	608
GGGAGCA G CAGGAAG	248	CUUCCUG CUGAUGAGGCCGUUAGGCCGAA IGGCUCC	609
GGGGACC C GACAGGC	249	GCCUGUC CUGAUGAGGCCGUUAGGCCGAA IGUCCCC	610
GGGUACC U GUGUGGA	251	UCCACAC CUGAUGAGGCCGUUAGGCCGAA IGUACCC	611

GGGUUCU U GGGAGCA	253	UGCUCCC CUGAUGAGGCCGUUAGGCCGAA IGAACCC	612
GGUACCU G UGUGGAA	255	UUCCACA CUGAUGAGGCCGUUAGGCCGAA IGGUACC	613
GGUCACA G UCUAUUA	258	UAAUAGA CUGAUGAGGCCGUUAGGCCGAA IGUGACC	614
GUACACA U GGAAUUA	261	UAAUUCC CUGAUGAGGCCGUUAGGCCGAA IGUGUAC	615
GUACCCA C AGACCCC	263	GGGGUCU CUGAUGAGGCCGUUAGGCCGAA IGGGUAC	616
GUAUGCC C CUCCAU	267	AUGGGAG CUGAUGAGGCCGUUAGGCCGAA IGCAUAC	617
GUCAGCA C AGUACAA	270	UUGUACU CUGAUGAGGCCGUUAGGCCGAA IGCUGAC	618
GUGUACC C ACAGACC	281	GGUCUGU CUGAUGAGGCCGUUAGGCCGAA IGUACAC	619
UAAAGCC A UGUGUAA	288	UUACACA CUGAUGAGGCCGUUAGGCCGAA IGCUUUA	620
UAACGCU G ACGGUAC	291	GUACCGU CUGAUGAGGCCGUUAGGCCGAA IGCGUUA	621
UACCACC G CUUGAGA	296	UCUCAAG CUGAUGAGGCCGUUAGGCCGAA IGUGGUA	622
UAGUGCA A CAGCAAA	303	UUUGCUG CUGAUGAGGCCGUUAGGCCGAA IGCACUA	623
UAUGCCC C UCCCAUC	310	GAUGGGA CUGAUGAGGCCGUUAGGCCGAA IGGCAUA	624
UCAGACC U GGAGGAG	321	CUCCUCC CUGAUGAGGCCGUUAGGCCGAA IGUCUGA	625
UGCAACU C ACAGUCU	338	AGACUGU CUGAUGAGGCCGUUAGGCCGAA IGUUGCA	626
UGCAUCA G AUGCUAA	339	UUAGCAU CUGAUGAGGCCGUUAGGCCGAA IGAUGCA	627
UGCCUCU U CAGCUAC	340	GUAGCUG CUGAUGAGGCCGUUAGGCCGAA IGAGGCA	628
UGGAACU U CUGGGAC	345	GUCCCAG CUGAUGAGGCCGUUAGGCCGAA IGUUCCA	629
UGGGUCA C AGUCUAU	354	AUAGACU CUGAUGAGGCCGUUAGGCCGAA IGACCCA	630
UGUACCC A CAGACCC	359	GGGUCUG CUGAUGAGGCCGUUAGGCCGAA IGGUACA	631
UGUGCCU C UUCAGCU	364	AGCUGAA CUGAUGAGGCCGUUAGGCCGAA IGGCACA	632
UGUUCCU U GGGUUCU	370	AGAACCC CUGAUGAGGCCGUUAGGCCGAA IGGAAACA	633
UGUJUGCA A CUCACAG	371	CUGUGAG CUGAUGAGGCCGUUAGGCCGAA IGCAACA	634
UUAGGCA G GGAAUACU	374	AGUAUCC CUGAUGAGGCCGUUAGGCCGAA IGCCUAA	635
UUGUGCA U CAGAUGC	389	GCAUCUG CUGAUGAGGCCGUUAGGCCGAA IGCACAA	636
UUUAACA U GUGGAAA	390	UUUCCAC CUGAUGAGGCCGUUAGGCCGAA IGUAAA	637

**Table VI: HIV env Target and G-cleaver Sequence**

Substrate	Seq ID	G-Cleaver Ribozyme	Seq ID
AACGCUG A CGGUACA	14	UGUACCG UGAUG GCAUGCACUAUGC GCG CAGCGUU	638
AAUAAACG C UGACGGU	22	ACCGUCA UGAUG GCAUGCACUAUGC GCG CGUUAUU	639
ACACAAUG C CUGUGUA	38	UACACAG UGAUG GCAUGCACUAUGC GCG CAUGUGU	640
ACCACCG C UUGAGAG	45	CUCUCAA UGAUG GCAUGCACUAUGC GCG CGGUGGU	641
ACCUGUG U GGAAAGA	49	UCUUUCC UGAUG GCAUGCACUAUGC GCG CACAGGU	642
AGAAGUG A AUUUAU	55	AUAUAAU UGAUG GCAUGCACUAUGC GCG CACUUCU	643
AGCAAUG U AUGCCCC	63	GGGGCAU UGAUG GCAUGCACUAUGC GCG CAUUGCU	644
AUGCCUG U GUACCCA	100	UGGGUAC UGAUG GCAUGCACUAUGC GCG CAGGCAU	645
AUGUAUG C CCCUCCC	104	GGGAGGG UGAUG GCAUGCACUAUGC GCG CAUACAU	646
CAAUUUG C UGAGGGC	125	GCCCCUA UGAUG GCAUGCACUAUGC GCG CAAAUUG	647
CAUAAUG A UAGUAGG	144	CCUACUA UGAUG GCAUGCACUAUGC GCG CAUUAUG	648
CCGCUUG A GAGACUU	159	AAGUCUC UGAUG GCAUGCACUAUGC GCG CAAGCGG	649
CUGGCUG U GGAAAGA	181	UCUUUCC UGAUG GCAUGCACUAUGC GCG CAGCCAG	650
GAGCCUG U GCCUCUU	208	AAGAGGC UGAUG GCAUGCACUAUGC GCG CAGGCUC	651
GCCUGUG C CUCUCA	227	UGAAGAG UGAUG GCAUGCACUAUGC GCG CACAGGC	652
GCCUGUG U ACCCACA	228	UGUGGGU UGAUG GCAUGCACUAUGC GCG CACAGGC	653
GUACCUG U GUGGAAA	264	UUUCCAC UGAUG GCAUGCACUAUGC GCG CAGGUAC	654
UAACAAUG U GGAAAAA	290	UUUUUCC UGAUG GCAUGCACUAUGC GCG CAUGUUA	655
UACAAUG U ACACAAUG	294	CAUGUGU UGAUG GCAUGCACUAUGC GCG CAUUGUA	656
UAIUAGUG C AACAGCA	307	UGCUGUU UGAUG GCAUGCACUAUGC GCG CACUAUA	657
UAIUUUUG U GCAUCAG	316	CUGAUGC UGAUG GCAUGCACUAUGC GCG CAAAAUA	658
UCAGAAUG C UAAAGCA	322	UGCUUUA UGAUG GCAUGCACUAUGC GCG CAUCUGA	659
UUUGCUG A GGGCUAU	392	AUAGCCC UGAUG GCAUGCACUAUGC GCG CAGCAAA	660
UUUUGUG C AUCAGAU	395	AUCUGAU UGAUG GCAUGCACUAUGC GCG CACAAAA	661

Table VII: HIV env Target and Zinzyme Sequence

Substrate	Seq ID	Zinzyme	Seq ID
AAUAACG C UGACGGU	22	ACCGUCA GCCGAAAGGCCAGUGAGGUCU CGUUAUU	662
AAUAGAG U UAGGCAG	23	CUGCCUA GCCGAAAGGCCAGUGAGGUCU CUCUAUU	663
ACACAUG C CUGUGUA	38	UACACAG GCCGAAAGGCCAGUGAGGUCU CAUGUGU	664
ACCACCG C UUGAGAG	45	CUCUCAA GCCGAAAGGCCAGUGAGGUCU CGGUGGU	665
ACCUGUG U GGAAAGA	49	UCUUUCC GCCGAAAGGCCAGUGAGGUCU CACAGGU	666
AGCAAUG U AUGCCCC	63	GGGGCAU GCCGAAAGGCCAGUGAGGUCU CAUUGC	667
AGUUAGG C AGGGAUA	80	UAUCCU GCCGAAAGGCCAGUGAGGUCU CCUAACU	668
AUGAUAG U AGGAGGC	99	GCCUCUJ GCCGAAAGGCCAGUGAGGUCU CUAUCAU	669
AUGCCUG U GUACCCA	100	UGGGUAC GCCGAAAGGCCAGUGAGGUCU CAGGCAU	670
AUGGCAG U CUAGCAG	101	CUGCUAG GCCGAAAGGCCAGUGAGGUCU CUGCCAU	671
AUGUAUG C CCCUCCC	104	GGGAGGG GCCGAAAGGCCAGUGAGGUCU CAUACAU	672
AUGUCAG C ACAGUAC	105	GUACUGU GCCGAAAGGCCAGUGAGGUCU CUGACAU	673
CAAUUUG C UGAGGGC	125	GCCCUCA GCCGAAAGGCCAGUGAGGUCU CAAAUUG	674
CAGGAAG C ACUAUGG	138	CCAUAGU GCCGAAAGGCCAGUGAGGUCU CUUCCUG	675
CCUAAAG C CAUGUGU	160	ACACAUG GCCGAAAGGCCAGUGAGGUCU CUUUAGG	676
CCUUGGG U UCUUGGG	166	CCCAAGA GCCGAAAGGCCAGUGAGGUCU CCCAAGG	677
CUCACAG U CUGGGGC	175	GCCCCAG GCCGAAAGGCCAGUGAGGUCU CUGUGAG	678
CUCCAGG C AAGAGUC	176	GACUCUU GCCGAAAGGCCAGUGAGGUCU CCUGGAG	679
CUGACGG U ACAGGCC	179	GGCCUGU GCCGAAAGGCCAGUGAGGUCU CCGUCAG	680
CUGGCUG U GGAAAGA	181	UCUUUCC GCCGAAAGGCCAGUGAGGUCU CAGCCAG	681
CUUJUGAG C CAAUUCC	191	GGAAUUG GCCGAAAGGCCAGUGAGGUCU CUCAAAG	682
GAGCCUG U GCCUCUU	208	AAGAGGC GCCGAAAGGCCAGUGAGGUCU CAGGCUC	683
GCAAGAG U CCUGGCU	217	AGCCAGG GCCGAAAGGCCAGUGAGGUCU CUCUUGC	684
GCCUGUG C CUCUCA	227	UGAAGAG GCCGAAAGGCCAGUGAGGUCU CACAGGC	685
GCCUGUG U ACCCACA	228	UGUGGGU GCCGAAAGGCCAGUGAGGUCU CACAGGC	686
GCUGUGG U AUAAUAA	233	UUUUAU GCCGAAAGGCCAGUGAGGUCU CCACAGC	687
GGAGAAG U GAAUUAU	237	AUAAUJC GCCGAAAGGCCAGUGAGGUCU CUUCUCC	688
GGAGCAG C AGGAAGC	238	GUUCCU GCCGAAAGGCCAGUGAGGUCU CUGCUCC	689
GGUAUAG U GCAACAG	256	CUGUUGC GCCGAAAGGCCAGUGAGGUCU CUAUACC	690
GUACAGG C CAGACAA	262	UUGUCUG GCCGAAAGGCCAGUGAGGUCU CCUGUAC	691
GUACCUG U GUGGAAA	264	UUUCCAC GCCGAAAGGCCAGUGAGGUCU CAGGUAC	692
GUCACAG U CUAUUAU	269	AUAAUAG GCCGAAAGGCCAGUGAGGUCU CUGUGAC	693
UAACAAUG U GGAAAAAA	290	UUUUUCC GCCGAAAGGCCAGUGAGGUCU CAUGUUA	694
UAAUCAG U UUAUGGG	292	CCCAUAA GCCGAAAGGCCAGUGAGGUCU CUGAUUA	695
UACAAUG U ACACAUG	294	CAUGUGU GCCGAAAGGCCAGUGAGGUCU CAUUGUA	696
UAUAGUG C AACAGCA	307	UGCUGUU GCCGAAAGGCCAGUGAGGUCU CACUAUA	697
UAUGGGG U ACCUGUG	311	CACAGGU GCCGAAAGGCCAGUGAGGUCU CCCCAUA	698
UAUUUUG U GCAUCAG	316	CUGAUGC GCCGAAAGGCCAGUGAGGUCU CAAAAUA	699
UCAACAG C UCCUAGG	317	CCUAGGA GCCGAAAGGCCAGUGAGGUCU CUGUUGA	700
UCAGAUG C UAAAGCA	322	UGCUUUA GCCGAAAGGCCAGUGAGGUCU CAUCUGA	701
UCUJUCAG C UACCACC	332	GGUGGUUA GCCGAAAGGCCAGUGAGGUCU CUGAAGA	702
UGUCUGG U AUAGUGC	362	GCACUAU GCCGAAAGGCCAGUGAGGUCU CCAGACA	703
UUGGGAG C AGCAGGA	386	UCCUGCU GCCGAAAGGCCAGUGAGGUCU CUCCCAA	704
UUUUGUG C AUCAGAU	395	AUCUGAU GCCGAAAGGCCAGUGAGGUCU CACAAAA	705

Table VIII: HIV env Target and DNAzyme Sequence

Substrate	Seq ID	DNAzyme	Seq ID
AAAAUAU U UCAUAAU	2	ATTATGA GGCTAGCTACAAACGA TATTTTT	706
AAAAGAA U GAACAAG	3	CTTGTTC GGCTAGCTACAAACGA TTCTTTT	707
AAAAUAA C AUGGUAG	4	CTACCAT GGCTAGCTACAAACGA TTATTTT	708
AAAGCCA U GUGUAAA	8	TTTACAC GGCTAGCTACAAACGA TGGCTTT	709
AACAUAG C CUGGAUG	12	CATCCAG GGCTAGCTACAAACGA TCATGTT	710
AAGUGAA U UAUUAUA	21	TTATATA GGCTAGCTACAAACGA TTCACTT	711
AAUAAACG C UGACGGU	22	ACCGTCA GGCTAGCTACAAACGA CGTTATT	712
AAUAGAG U UAGGCAG	23	CTGCCTA GGCTAGCTACAAACGA CTCTATT	713
AAUUUAU U AAAUAUA	31	TATATT GGCTAGCTACAAACGA TATAATT	714
ACAAUUA U UGUCUGG	36	CCAGACA GGCTAGCTACAAACGA TAATTGT	715
ACACAAUG C CUGUGUA	38	TACACAG GGCTAGCTACAAACGA CATGTGT	716
ACCACCG C UUGAGAG	45	CTCTCAA GGCTAGCTACAAACGA CGGTGGT	717
ACCCCAA C CCACAAAG	47	CTTGTGG GGCTAGCTACAAACGA TTGGGGT	718
ACCUGUG U GGAAAGA	49	TCTTTCC GGCTAGCTACAAACGA CACAGGT	719
ACGCUGA C GGUACAG	50	CTGTACC GGCTAGCTACAAACGA TCAGCGT	720
AGCAAUG U AUGCCCC	63	GGGGCAT GGCTAGCTACAAACGA CATTGCT	721
AGCACUA U GGGCGCA	64	TGCGCCC GGCTAGCTACAAACGA TAGTGCT	722
AGUACAA U GUACACA	77	TGTGTAC GGCTAGCTACAAACGA TTGTACT	723
AGUJAGG C AGGGUA	80	TATCCCT GGCTAGCTACAAACGA CCTAACT	724
AUAAAAAA U AUUCAUA	83	TATGAAT GGCTAGCTACAAACGA TTTTTAT	725
AUAAAUA U AAAGUAG	84	CTACTTT GGCTAGCTACAAACGA TATTTAT	726
AUAAUGA U AGUAGGA	87	TCCTACT GGCTAGCTACAAACGA TCATTAT	727
AUUAUAA U AUAAAGU	90	ACTTTAT GGCTAGCTACAAACGA TTTATAT	728
AUAUUCA U AAUGUA	93	TATCATT GGCTAGCTACAAACGA TGAATAT	729
AUGAUAG U AGGAGGC	99	GCCTCCT GGCTAGCTACAAACGA CTATCAT	730
AUGCCUG U GUACCCA	100	TGGGTAC GGCTAGCTACAAACGA CAGGCAT	731
AUGGCAG U CUAGCAG	101	CTGCTAG GGCTAGCTACAAACGA CTGCCAT	732
AUGUACA C AUGGAAU	103	ATTCCAT GGCTAGCTACAAACGA TGTACAT	733
AUGUAUG C CCCUCCC	104	GGGAGGG GGCTAGCTACAAACGA CATACAT	734
AUGUCAG C ACAGUAC	105	GTACTGT GGCTAGCTACAAACGA CTGACAT	735
AUUCCCA U ACAUUAU	111	ATAATGT GGCTAGCTACAAACGA TGGGAAT	736
AUUUUAA C AUGUGGA	115	TCCACAT GGCTAGCTACAAACGA TTAAAAT	737
CAACUCA C AGUCUGG	119	CCAGACT GGCTAGCTACAAACGA TGAGTTG	738
CAAUGUA C ACAUGGA	121	TCCATGT GGCTAGCTACAAACGA TACATTG	739
CAAUGUA U GCCCCUC	122	GAGGGGC GGCTAGCTACAAACGA TACATTG	740
CAAUUUG C UGAGGGC	125	GCCCTCA GGCTAGCTACAAACGA CAAATTG	741
CAGACAA U UAUUGUC	131	GACAATA GGCTAGCTACAAACGA TTGTCTG	742
CAGGAAG C ACUAUAG	138	CCATAGT GGCTAGCTACAAACGA CTTCCTG	743
CAGUCUA U UAUGGGG	142	CCCCATA GGCTAGCTACAAACGA TAGACTG	744
CAGUUUA U GGGAUCA	143	TGATCCC GGCTAGCTACAAACGA TAAACTG	745
CAUCAGA U GCUAAAG	146	CTTTAGC GGCTAGCTACAAACGA TCTGATG	746
CCACAGA C CCCAACCC	150	GGTTGGG GGCTAGCTACAAACGA TCTGTGG	747
CCAUACA U UAUUGUG	154	CACAATA GGCTAGCTACAAACGA TGTATGG	748
CCUAAAAG C CAUGUGU	160	ACACATG GGCTAGCTACAAACGA CTTTAGG	749
CCUUGGG U UCUUGGG	166	CCCAAGA GGCTAGCTACAAACGA CCCAAGG	750
CUCACAG U CUGGGGC	175	GCCCCAG GGCTAGCTACAAACGA CTGTGAG	751
CUCCAGG C AAGAGUC	176	GACTCTT GGCTAGCTACAAACGA CCTGGAG	752
CUGACGG U ACAGGCC	179	GGCCTGT GGCTAGCTACAAACGA CCGTCAG	753
CUGGCUG U GGAAAGA	181	TCTTTCC GGCTAGCTACAAACGA CAGCCAG	754
CUGUGUA C CCACAGA	186	TCTGTGG GGCTAGCTACAAACGA TACACAG	755

CUUCAGA C CUGGAGG	187	CCTCCAG GGCTAGCTACAACGA TCTGAAG	756
CUUUGAG C CAAUUCC	191	GGAATTG GGCTAGCTACAACGA CTCAAAG	757
GACGGUA C AGGCCAG	203	CTGGCCT GGCTAGCTACAACGA TACCGTC	758
GAGCCUG U GCCUCUU	208	AAGAGGC GGCTAGCTACAACGA CAGGCTC	759
GAUUAUA U CAGUUUA	213	TAAACTG GGCTAGCTACAACGA TTATATC	760
GCAAGAG U CCUGGCCU	217	AGCCAGG GGCTAGCTACAACGA CTCTTGC	761
GCAGGGGA U ACUCACC	222	GGTGAGT GGCTAGCTACAACGA TCCCTGC	762
GCCUGUG C CUCUUCA	227	TGAAGAG GGCTAGCTACAACGA CACAGGC	763
GCCUGUG U ACCCACA	228	TGTGGGT GGCTAGCTACAACGA CACAGGC	764
GCUGUGG U AUAUAAA	233	TTTATAT GGCTAGCTACAACGA CCACAGC	765
GGAAAAA U AACAUAGG	234	CCATGTT GGCTAGCTACAACGA TTTTTCC	766
GGAAGCA C UAUGGGC	235	GCCCATA GGCTAGCTACAACGA TGCTTCC	767
GGAGAAG U GAAUUAU	237	ATAATTC GGCTAGCTACAACGA CTTCTCC	768
GGAGCAG C AGGAAGC	238	GCTTCCT GGCTAGCTACAACGA CTGCTCC	769
GGAUCAA C AGCUCCU	241	AGGAGCT GGCTAGCTACAACGA TTGATCC	770
GGGACAA U UGGAGAA	247	TTCTCCA GGCTAGCTACAACGA TTGTCCC	771
GGUAUAG U GCAACAG	256	CTGTTGC GGCTAGCTACAACGA CTATACC	772
GUACACA U GGAAUUA	261	TAATTCC GGCTAGCTACAACGA TGTGTAC	773
GUACAGG C CAGACAA	262	TTGTCTG GGCTAGCTACAACGA CCTGTAC	774
GUACCCA C AGACCCC	263	GGGGTCT GGCTAGCTACAACGA TGGGTAC	775
GUACCUG U GUGGAAA	264	TTTCCAC GGCTAGCTACAACGA CAGGTAC	776
GUCACAG U CUAAUUAU	269	ATAATAG GGCTAGCTACAACGA CTGTGAC	777
GUCAGCA C AGUACAA	270	TTGTACT GGCTAGCTACAACGA TGCTGAC	778
GUUGCAA C UCACAGU	285	ACTGTGA GGCTAGCTACAACGA TTGCAAC	779
UAACAUAG U GGAAAAA	290	TTTTTCC GGCTAGCTACAACGA CATGTTA	780
UAAUCAG U UUAUGGG	292	CCCATAA GGCTAGCTACAACGA CTGATTA	781
UACAAUAG U ACACAUG	294	CATGTGT GGCTAGCTACAACGA CATTGTA	782
UAUAGUG C AACAGCA	307	TGCTGTT GGCTAGCTACAACGA CACTATA	783
UAUGGGG U ACCUGUG	311	CACAGGT GGCTAGCTACAACGA CCCCCATA	784
UAUUUUG U GCAUCAG	316	CTGATGC GGCTAGCTACAACGA CAAAATA	785
UCAACAG C UCCUAGG	317	CCTAGGA GGCTAGCTACAACGA CTGTTGA	786
UCAAUAA C GCUGACG	318	CGTCAGC GGCTAGCTACAACGA TTATTGA	787
UCAGAUG C UAAAGCA	322	TGCTTTA GGCTAGCTACAACGA CATCTGA	788
UCCCAUA C AUUAUUG	327	CAATAAT GGCTAGCTACAACGA TATGGGA	789
UCUAAUA U GGGGUAC	330	GTACCCCC GGCTAGCTACAACGA TAATAGA	790
UCUGGUA U AGUGCAA	331	TTGCACT GGCTAGCTACAACGA TACCAGA	791
UCUUCAG C UACCACC	332	GGTGGTA GGCTAGCTACAACGA CTGAAGA	792
UGAAUUA U AUAAAUA	334	TATTTAT GGCTAGCTACAACGA TAATTCA	793
UGAGGGGA C AAUUGGA	336	TCCAATT GGCTAGCTACAACGA TCCCTCA	794
UGGGGUAC C CUGUGUG	353	CACACAG GGCTAGCTACAACGA TACCCCA	795
UGGGUCA C AGUCUAU	354	ATAGACT GGCTAGCTACAACGA TGACCCA	796
UGGUUAU A AAAAUA	357	TATTTTT GGCTAGCTACAACGA TATACCA	797
UGUCUGG U AUAGUGC	362	GCACTAT GGCTAGCTACAACGA CCAGACA	798
UGUGGAA C UUCUGGG	367	CCCAGAA GGCTAGCTACAACGA TTCCACA	799
UGUGGUA U AUAAAAA	368	TTTTTAT GGCTAGCTACAACGA TACCACA	800
UUAAGAA U AGUUUUU	373	AAAAACT GGCTAGCTACAACGA TTCTTAA	801
UUCAUAA U GAUAGUA	379	TACTATC GGCTAGCTACAACGA TTATGAA	802
UUGGGAG C AGCAGGA	386	TCCTGCT GGCTAGCTACAACGA CTCCCCAA	803
UUGUGCA U CAGAUGC	389	GCATCTG GGCTAGCTACAACGA TGCACAA	804
UUUAACA U GUGGAAA	390	TTTCCAC GGCTAGCTACAACGA TGTTAAA	805
UUUUGUG C AUCAGAU	395	ATCTGAT GGCTAGCTACAACGA CACAAAAA	806

**Table IX: HIV env Target and Amberzyme Sequence**

Substrate	Seq ID	Amberzyme	Seq ID
AACGGUG A CGGUACA	14	UGUACCG CGAGGAACUCC CU UCAAGGACAUUCGUCCCC CGCGGU	807
AAUAAUC C UGACGGU	22	ACCGGUCA GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CGUUAU	808
AAUAGAG U UAGGCAG	23	CGGCCUA GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUCUAU	809
ACAAUUG G AGAAGUG	37	CACUUUCU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAAUUGU	810
ACACAUG C CUGUGUA	38	UACACAG GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAUGUGU	811
ACAUGUG G AAAAUAA	44	UAUUUUU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CACAUUGU	812
ACCACCG C UTGAGAG	45	CUCUCAA GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CGGGGGU	813
ACCUUGU U GGAAGAA	49	UCUUUCU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CACAGGU	814
AGAAAGUG A AUUAUAU	55	AUAUAAU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CACUUCU	815
AGACCUUG G AGGAGGA	58	UCCUCCU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAGGCU	816
AGCAAUG U AUGCCCC	63	GGGGCAU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAUUGC	817
AGGCAAG A GUCCUGG	74	CCAGGAC GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUTGCCU	818
AGGCAGG G AUACUCA	75	UGAGUAU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CCUGCCU	819
AGUUAGG C AGGGAUJA	80	UAUCCCU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CCUAACU	820
AUAUAGAG G GACAUU	92	AUJUGUC GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUCAUAU	821
AUGAGGG A CAAUUGG	98	CCAAUUG GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CCCUCAU	822
AUGAUAG U AGGGGC	99	GCCUCCU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUAUCAU	823
AUGCCUG U GUACCCA	100	UGGGUAC GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAGGCAU	824
AUGGCAG U CUGGAG	101	CUGCUAG GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUGCCAU	825
AUUAUAG C CCCUCCC	104	GGGGGG GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAAUAAU	826
AUUGGAG A AGUGAAU	112	AUUCACU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUCCAAU	827
AUGUCAG C ACAGUAC	105	GUACUGU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUGACAU	828
AUUAUAG G GUACCUG	109	CAGGUAC GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAAUAAU	829
CAAUUGG A GAAGUGA	124	UACACU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUCCAAU	830
CAAUUGG C UGAGGGC	117	CCACUCU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CCAUUTG	831
CAUCUAG G GCGCAGC	125	GCCCCUCA GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAAUUG	832
CAGCAGG A AGCACUA	136	UAGUGCU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CCUGCUG	833
CAGGAAG C ACUAUGG	138	CCAUAGU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CUCUCCUG	835
CAUAAUAG A UAGUAGG	144	CCUACUA GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CAUUAUG	836
CAUGUGG A AAAUAAA	148	UUAUUTU GGAGGAACUCC CU UCAAGGACAUUCGUCCCC CCAACAUG	837

CCACAG A CCCAAC	156	GUUGGGG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUGUGGG	838
CCGUUG A GAGACU	159	AAGUCUC GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CAAGCGG	839
CCUAG C CAUGUG	160	ACACAUG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CTTUAGG	840
CCUGGAG G AGGAGA	162	AUCUCU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUCCAGG	841
CCUUGGG U UCUGGG	166	CCCAAGA GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCCAAGG	842
CUAAAGG A UCAACAG	171	CUGUUGA GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCUUAG	843
CUAGUUG G AGUAAA	172	UAUUACU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CAACUAG	844
CUCACAG U CUGGGC	175	GCCCCAG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUGUGAG	845
CUCCAG C AAGAGUC	176	GACUCU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCUGGAG	846
CUGACGG U ACAGGC	179	GGCCUGU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCGUAG	847
CUUAGGG A GGAGAU	180	UAUCUCC GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCUCCAG	848
CUGGCUG U GGAAGA	181	UCUUUCU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CAGCCAG	849
CUUUGAG C CAAUUC	191	GGAAUTG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUCAAAG	850
GAAGAAG A AGGUGGA	194	UCCACCU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUUUUUC	851
GAAGAAG G UGGAGAG	195	CUCUCCA GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUUUUUC	852
GACCUGG A GGAGGAG	202	CUCCUCU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCAGGUC	853
GAGCCUG U GCCUCUU	208	AGAGGG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CAGGCUC	854
GAGGAG G GAGAUGA	209	UCAUAUC GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCUCCUC	855
GAGUUAG G CAGGGAU	211	AUCCUCU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUAACUC	856
GCAAGAG U CCUGGU	217	AGCCAGG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUCUUGC	857
GCAGGAG G AAGCACU	220	AGUGCUU GGAGGUU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUGCUGC	858
GCAUCAG A UGCUAAA	223	UUUAGCA GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUGAUGC	859
GCCUGUG C CUCUUCA	227	UGAAGAG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CACAGGC	860
GCCUGUG U ACCCACA	228	UGUGGGU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CACAGGC	861
GCUCUCC G CAAGAGU	229	ACUCUUG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUGGAGC	862
GCUCUCC G AAACUCA	230	UGAGUUT GGAGGUU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCAAGGC	863
GCUGACG G UACGGC	231	GUAAUUC GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CGUCAGC	864
GCUGUGG A AAGAUAC	232	GUAUUUCU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CCAACAGC	865
GCUGUGG U AUUAAA	233	UUUUAU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUCUCCC	866
GGAGGAAG U GAAUUAU	237	AUAAUUC GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUCUCCC	867
GGAGGGG A UACUCAC	238	GCUCUCCU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUGCUCC	868
GGAGGAG C AGGAAGC	240	CAUACU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUCUCCC	869
GGAGGAG G AGAUAG	243	GUGAGUA GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUCUCCC	870
GGCAGGG A UACUCAC	245	UAUUCUU GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CACAGCC	871
GGCUGUG G AAAGAU	246	UUUUAUA GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CACAGCC	872
GGUACAG G CCAGACA	254	UGUCUGG GGAGGAACUCC CU UCAAGGACAUCGUCCCCG CUGUACC	873

GGUAUAG U GCAACAG	256	CUGUUGC CGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CUUACC	874
GUACAGG C CAGACAA	262	UUGUCUG GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCUGUAC	875
GUACCUG U GUCCAAA	264	UUUCCAC CGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAGGUAC	876
GUACACG U CUAUUAU	269	AUAAUAG GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CUGUGAC	877
GUUCUUG G GAGCAGC	284	GCUGUC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAAGAAC	878
GUUUAUG G GAUCAAA	286	UUUGUAC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAUAAAC	879
UAACAU G GGAAGAA	290	UUUUUCC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAUGUUA	880
UAUUCAG U UUAUGGG	292	CCCAUAA GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CUGAUUA	881
UACAAUG U ACACAU	294	CAUGUGU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAUUGUA	882
UAGGCG G GAUCUC	302	GAUGUAC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CGGCCUA	883
UAGUUGG A GUAAUAA	304	UUUUUAC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCAACUA	884
UAUAGUG C AACAGCA	307	UGCUGUU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CACUUA	885
UAUGAGG G ACAAUUG	309	CAAUUGU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCUCAUUA	886
UAUGGGG U ACCUGUG	311	CACAGGU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCCCAUA	887
UAUUAUG G GGUACCU	313	AGGUACC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAUAAUA	888
UAUUUGG U GCAUCAG	316	CUGAUGC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAAAUAA	889
UCAACAG C UCCUAGG	317	CCUAGGA GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CUGUUGA	890
UCAGAUG C UAAAGCA	322	UGCUUUA GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAUCUGA	891
UCCUUGG G UUCUUGG	329	CCAAGAA GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCAAGGA	892
UCUUCAG C UACCACC	332	GGUGGUU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CUGAAGA	893
UCUTUGGG A GCAAGCAG	333	CUGCUGC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCCAAGA	894
UGCUCUG C AAAACUC	342	GAGUUTU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAGAGCA	895
UGGAAGG A UACCUAA	344	UAGGUU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CUUCCA	896
UGUCUGG U AUAGUGC	362	GCACUAU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCAGACA	897
UUAUUGG G UACCUGU	376	ACAGGUU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCCAUAA	898
UUCUUGG G GUUCUJG	381	CAAGAAC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAAGGAA	899
UUCUUGG G AGCAGCA	382	UGCUGGU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCAAGAA	900
UUGGGAG C AGCAGGA	386	UCCUGGU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CUCCCAA	901
UUGUCUG G UAUAGUG	388	CAUCUUA GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAGACAA	902
UUUAUGG G AUCAAAG	391	CUUUGAU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CCAUAAA	903
UUUGCUG A GGGCUAU	392	AUAGCCC GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CAGCAAA	904
UUUUGUG C AUCAGAU	395	AUCUGAU GGAGGAAACUCC CU UCAAGGACAUUCGUCCCCG CACAAAA	905

**Table X: HIV env Target and Antisense Sequence**

Sequence	Seq ID	Antisense	Seq ID
CAGCAGGAAGCACUAUGGGCG	396	CGCCCATAGTGCTTCCTGCTG	906
AGCAGGAAGCACUAUGGGCGC	397	GCGCCCATAGTGCTTCCTGCT	907
GCAGCAGGAAGCACUAUGGGC	398	GCCCATAGTGCTTCCTGCTGC	908
AGCAGCAGGAAGCACUAUGGG	399	CCCATAGTGCTTCCTGCTGCT	909
GAGCAGCAGGAAGCACUAUGG	400	CCATAGTGCTTCCTGCTGCTC	910
GGAGCAGCAGGAAGCACUAUG	401	CATAGTGCTTCCTGCTGCTCC	911
CGCUGACGGUACAGGCCAGAC	402	GTCTGGCCTGTACCGTCAGCG	912
ACAAUUGGAGAAGUGAAUUAU	403	ATAATTCACTTCTCCAATTGT	913
ACCGUGACGGUACAGGCCAGA	404	TCTGGCCTGTACCGTCAGCGT	914
AGUUAGGCAGGGAUACUCACC	405	GGTGAGTATCCCTGCCTAACT	915
CAAUUGGAGAAGUGAAUUAUA	406	TATAATTCACTTCTCCAATTG	916
GAGUUAGGCAGGGAUACUCAC	407	GTGAGTATCCCTGCCTAACTC	917
AGAGUUAGGCAGGGAUACUCA	408	TGAGTATCCCTGCCTAACTCT	918
AUUGGAGAAGUGAAUUAUAUA	409	TATATAATTCACTTCTCCAAT	919
AAUUGGAGAAGUGAAUUAUAU	410	ATATAATTCACTTCTCCAATT	920
GACAAUUGGAGAAGUGAAUUA	411	TAATTCACTTCTCCAATTGTC	921
UUGGAGAAGUGAAUUAUAUA	412	TTATATAATTCACTTCTCCAA	922
UAGAGUUAGGCAGGGAUACUC	413	GAGTATCCCTGCCTAACTCTA	923
UGCCUGUGUACCCACAGACCC	414	GGGTCTGTGGGTACACAGGC	924
AUGCCUGUGUACCCACAGACC	415	GGTCTGTGGGTACACAGGCAT	925
AUAGAGUUAGGCAGGGAUACU	416	AGTATCCCTGCCTAACTCTAT	926
CAUGCCUGUGUACCCACAGAC	417	GTCTGTGGGTACACAGGCATG	927
AAUAGAGUUAGGCAGGGAUAC	418	GTATCCCTGCCTAACTCTATT	928
ACACAUGCCUGUGUACCCACA	419	TGTGGGTACACAGGCATGT	929
CACAUGCCUGUGUACCCACAG	420	CTGTGGGTACACAGGCATGT	930
ACAUGCCUGUGUACCCACAGA	421	TCTGTGGGTACACAGGCATGT	931
GGACAAUUGGAGAAGUGAAUU	422	AATTCACTTCTCCAATTGTC	932
AGCAAUGUAUGCCCCUCCAU	423	ATGGGAGGGGCATACATTGCT	933
GCUGACGGUACAGGCCAGACA	424	TGTCTGGCCTGTACCGTCAGC	934
GCCUGUGUACCCACAGACCCC	425	GGGGTCTGTGGGTACACAGGC	935
UAUUAUGGGGUACCUGUGUGG	426	CCACACAGGTACCCATAATA	936
GCUCCAGGCAAGAGUCCUGGC	427	GCCAGGACTCTGCCTGGAGC	937
CAGCUCCAGGCAAGAGUCCUG	428	CAGGACTCTGCCTGGAGCTG	938
AGCUCCAGGCAAGAGUCCUGG	429	CCAGGACTCTGCCTGGAGCT	939
CUCCAGGCAAGAGUCCUGGU	430	AGCCAGGACTCTGCCTGGAG	940
CCUGUGUACCCACAGACCCC	431	TGGGGTCTGTGGGTACACAGG	941
CUGACGGUACAGGCCAGACAA	432	TTGTCTGGCCTGTACCGTCAG	942
CCAAUUCCAUACAUUAUJGU	433	ACAATAATGTATGGGAATTGG	943
AUUAUGGGGUACCUGUGUGGA	434	TCCACACAGGTACCCATAAT	944
UACCCACAGACCCCAACCCAC	435	GTGGGTTGGGGTCTGTGGGT	945
UGUCUGGUAUAGUGCAACAGC	436	GCTGTTGCACTATACCAGACA	946
CUUUGGGAGCAGCAGGAAGCA	437	GTGCTTCCTGCTGCTCCAAAG	947
UCUUGGGAGCAGCAGGAAGCA	438	TGCTTCCTGCTGCTCCAAAGA	948
GUCUGGUAUAGUGCAACAGCA	439	TGCTGTTGCACTATACCAGAC	949
GUACCCACAGACCCCAACCC	440	TGGGGTTGGGGTCTGTGGGTAC	950
UUCUUGGGAGCAGCAGGAAGC	441	GCTTCCTGCTGCTCCAAAGAA	951
UGACGGUACAGGCCAGACAAU	442	ATTGTCTGGCCTGTACCGTC	952
UGGCUGUGGUAUUAUAAAUA	443	TATTTTATATACCAACAGCCA	953
UGUGCCUCUUCAGCUACCACC	444	GGTGGTAGCTGAAGAGGCCAA	954
GACGGUACAGGCCAGACAAU	445	AATTGTCTGGCCTGTACCGTC	955
UGUGUACCCACAGACCCCAAC	446	GTTGGGGTCTGTGGGTACACA	956

UGGGGUACCUGUGUGGAAAGA	447	TCTTCCACACAGGTACCCCA	957
GUGUACCCACAGACCCCAACC	448	GGTTGGGTCTGTGGGTACAC	958
UAUGGGGUACCUGUGUGGAAA	449	TTTCCACACAGGTACCCATA	959
AUGGGGUACCUGUGUGGAAAG	450	CTTCCACACAGGTACCCAT	960
GGCUGUGGUUAUAAAAAUAU	451	ATATTTTATATACACAGCC	961
UGUACCCACAGACCCCAACCC	452	GGGTTGGGTCTGTGGGTACA	962
CCCACAGACCCCAACCCACAA	453	TTGTGGGTGGGGTCTGTGGG	963
CUGUGCCUCUUCAGCUACCAC	454	GTGGTAGCTGAAGAGGCACAG	964
GCUGUGGUUAUAAAAAUAUU	455	AATATTTTATATACACAGC	965
CCACAGACCCCAACCCACAAG	456	CTTGTGGGTGGGGTCTGTGG	966
GUUCUUGGGAGCAGCAGGAAG	457	CTTCCTGCTGCTCCAAAGAAC	967
ACCCACAGACCCCAACCCACA	458	TGTGGGTTGGGGTCTGTGGGT	968
CACAGACCCCAACCCACAAGA	459	TCTTGTGGGTGGGGTCTGTG	969
CCUGUGCCUCUUCAGCUACCA	460	TGGTAGCTGAAGAGGCACAGG	970
ACAGACCCCAACCCACAAGAA	461	TTCTTGTGGGTGGGGTCTGT	971
GGUUCUUGGGAGCAGCAGGAA	462	TTCCCTGCTGCTCCAAAGAAC	972
GCAACUCACAGUCUGGGCAU	463	ATGCCCCAGACTGTGAGTTGC	973
UGCAACUCACAGUCUGGGCA	464	TGCCCCAGACTGTGAGTTGCA	974
GGGUUCUUGGGAGCAGCAGGA	465	TCCTGCTGCTCCAAAGAACCC	975
UUGCAACUCACAGUCUGGGC	466	GCCCCAGACTGTGAGTTGCAA	976
AUGAGGGACAIIJUGGAGAAGU	467	ACTTCTCCAATTGTCCCTCAT	977
UGUUGCAACUCACAGUCUGGG	468	CCCAGACTGTGAGTTGCAACA	978
UGAGGGACAAUUGGAGAAGUG	469	CACTTCTCCAATTGTCCCTCA	979
UGAAUUAUAAAUAUAAAAGU	470	ACTTTATATTATATAATTCA	980
GUUGCAACUCACAGUCUGGG	471	CCCCAGACTGTGAGTTGCAAC	981
UGGAGAAGUGAAUUAUAAA	472	TTTATATAATTCACTTCTCCA	982
UUGGGUUCUUGGGAGCAGCAG	473	CTGCTGCTCCAAAGAACCCAA	983
AAAGCCUAAAGCCAUGUGUAA	474	TTACACATGGCTTTAGGCTTT	984
UGGGUUCUUGGGAGCAGCAGG	475	CCTGCTGCTCCAAAGAACCCAA	985
GGAGAAGUGAAUUAUAAA	476	ATTTATATAATTCACTTCTCC	986
GAGAAGUGAAUUAUAAAUA	477	TATTTATATAATTCACTTCTC	987
AGGGACAAUJUGGAGAAGUGAA	478	TTCACCTCTCCAATTGTCCCT	988
AAGUGAAUUAUAAAUAUAA	479	TTATTTATATAATTCACTT	989
GAGGGACAAUUGGAGAAGUGA	480	TCACCTCTCCAATTGTCCCTC	990
AUAUGAGGGACAAUUGGAGAA	481	TTCTCCAATTGTCCCTCATAT	991
AGAAGUGAAUUAUAAAUAU	482	ATATTATATAATTCACTTCT	992
CUGUGUACCCACAGACCCAA	483	TTGGGGTCTGTGGGTACACAG	993
GAAGUGAAUUAUAAAUAU	484	TATTTATATAATTCACTTC	994
UACAAUGUACACAUGGAAU	485	TAATTCCATGTGTACATTGTA	995
CAGUACAAUGUACACAUGGAA	486	TTCCATGTGTACATTGTACTG	996
AAGCCUAAAGCCAUGUGAAA	487	TTTACACATGGCTTTAGGCTT	997
CCUUGGGUUCUUGGGAGCAG	488	GCTGCTCCAAAGAACCCAAAGG	998
AGUACAAUGUACACAUGGAAU	489	ATTCCATGTGTACATTGTACT	999
UCAAUAACGUGACGGUACAG	490	CTGTACCGTCAGCGTTATTGA	1000
ACAUGUGGAAAAUAACAUGG	491	CCATGTTATTTTCCACATGT	1001
UCCUUGGGUUCUUGGGAGCAG	492	CTGCTCCAAAGAACCCAAAGGA	1002
ACGGUACAGGCCAGACAAUUA	493	TAATTGTCTGGCCTGTACCGT	1003
GUACAAUGUACACAUGGAAU	494	AATTCCATGTGTACATTGTAC	1004
UUGUCUGGUUAUGUGAACAG	495	CTGTTGCACTATACCAAGACAA	1005
UAAUCAGUUUAUGGAAUAAA	496	TTTGATCCATAAACTGATTA	1006
AGUGAAUUAUAAAUAUAAA	497	TTTATATTATATAATTCACT	1007
GGGACAAUUGGAGAAGUGAAU	498	ATTCACTTCTCCAATTGTCCC	1008
UUAUGGGGUACCUGUGUGGAA	499	TTCCACACAGGTACCCATAA	1009
AAGCAAUGUAUGCCCCUCCCA	500	TGGGAGGGGCATACATGCTT	1010

AAUCAGUUUAUGGGAUCAAAG	501	CTTGATCCCATAAACTGATT	1011
CAAUAACGCUGACGGUACAGG	502	CCTGTACCGTCAGCGTTATTG	1012
GUGCCUCUUCAGCUACCACCG	503	CGGTGGTAGCTGAAGAGGCAC	1013
GAUUAUAUCAGUUUAUGGGAU	504	ATCCCATAAACTGATTATATC	1014

Table XI: HIV env Target and siRNA Sequence

Sequence	Seq ID	sirNA + strand	Seq ID	sirNA - strand	Seq ID
CAGCAGGAAGGCACUAUGGCG	396	CAGCAGGAAGGCACUAUGGCGTT	1015	CGCCCCAUAGUGCUUCCUGUGTT	1124
AGCAGGAAGCACUAUGGGCC	397	AGCAGGAAGCACUAUGGGCGTT	1016	GGGCCCAUAGUGCUUCCUGUGTT	1125
GCAGCAGGAAGCACUAUGGGC	398	GCAGCAGGAAGCACUAUGGGCTT	1017	GCCCAUAGUGCUUCCUGUGTT	1126
AGCAGCAGGAAGCACUAUGGG	399	AGCAGCAGGAAGCACUAUGGGTT	1018	CCCAUAGUGCUUCCUGUGTT	1127
GAGCAGCAGGAAGCACUAUGG	400	GAGCAGCAGGAAGCACUAUGGGT	1019	CCAUAGUGCUUCCUGUGTT	1128
GGAGCAGCAGGAAGCACUAUG	401	GGAGCAGCAGGAAGCACUAUGTT	1020	CAUAGUGCUUCCUGUGCGCUCCTT	1129
CGCUGACGGUACAGGCCAGAC	402	CGCUGACGGUACAGGCCAGACTT	1021	GUCCGGCCUGUACCGGUACGGTT	1130
ACAAUUGGAGAAGUGAAUUAU	403	ACAAUUGGAGAAGUGAAUUAUTT	1022	AUAAAUUCACTUCUCCAAUUGUTT	1131
ACGGCUGACGGUACAGGCCAGA	404	ACGGCUGACGGUACAGGCCAGATT	1023	UCUJGGCCUGUACCGGUACGGTT	1132
AGUUUAGGCAGGGAUACUCACC	405	AGUUUAGGCAGGGAUACUCACCTT	1024	GGUGAGUAUCCUGCCUAACUTT	1133
CAAUUGGAGAAGUGAAUUAUA	406	CAAUUGGAGAAGUGAAUUAUATT	1025	UAAUAAUUCACUUCCUCAAUUGTT	1134
GAGUUAGGCAGGGAUACUCAC	407	GAGUUAGGCAGGGAUACUCACTT	1026	GUAGUAUCCUGCCUAACUTT	1135
AGAGUUAGGCAGGGAUACUCA	408	AGAGUUAGGCAGGGAUACUCATT	1027	UGAGUAUCCUGCCUAACUTT	1136
AGUUGGAGAAGUGAAUUAUA	409	AGUUGGAGAAGUGAAUUAUATT	1028	UAUAAAUUUCACUUCUCCAAUUTT	1137
AAUUGGAGAAGUGAAUUAUAU	410	AAUUGGAGAAGUGAAUUAUATT	1029	AUAAAUUUCACUUCUCCAAUUTT	1138
GACAAUUGGAGAAGUGAAUUA	411	GACAAUUGGAGAAGUGAAUATT	1030	UAUAAAUCUUCUCCAAUUGUTT	1139
UUGGAGAAGUGAAUUAUAUA	412	UUGGAGAAGUGAAUUAUAAATT	1031	UTUAAAUAUUCACUUCCAAUTT	1140
AAUUGGAGAAGUGAAUUAUAU	413	AAUUGGAGAAGUGAAUUAUATT	1032	GAGUAUCCUGCCUAACUCUATT	1141
UAGAGUUAGGCAGGGAUACUC	414	UAGAGUUAGGCAGGGAUACUC	1033	GGUCUGUGGGUACACAGGCATT	1142
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AUGCCUGUGGUACCCACAGACC	416	AUGCCUGUGGUACCCACAGACCTT	1035	AGUAUCCUGCCUAACUCUATT	1144
AUAGAGUUAGGCAGGGAUACU	417	AUAGAGUUAGGCAGGGAUACUTT	1036	GUUCUGGGGUACACAGGCAUGTT	1145
CAUGCCUGUGGUACCCACAGAC	418	AAUAGAGUUAGGCAGGGAUACTT	1037	GUAUCCCCUGCCUAACUCUATT	1146
AAUAGAGUUAGGCAGGGAUAC	419	AAUAGAGUUAGGCAGGGAUAC	1038	UGUGGGGUACACAGGCAUGUGUTT	1147
ACACAUGCCUGUGUACCCACA	420	ACACAUGCCUGUGUACCCACAGT	1039	CUGUGGGGUACACAGGCAUGUTT	1148
CACAUAGCCUGUGUACCCACAG	421	CACAUAGCCUGUGUACCCACAGTT	1040	UCUGUGGGGUACACAGGCAUGUTT	1149
ACAUAGCCUGUGUACCCACAGA	422	ACAUAGCCUGUGUACCCACAGA	1041	AAUUCACUUCUCCAAUUGUCUTT	1150
GGACAAUUGGAGAAGUGAAUU	423	GGACAAUUGGAGAAGUGAAUUTT	1042	AUGGGAGGGGGCAUACAUUGUTT	1151
AGCAAAUGGUAGCCCCUCCAU	424	AGCAAAUGGUAGCCCCUCCAU	1043	UGUCUGGGCCUGUACCCGUCAGCTT	1152
GCGACGGGUACAGGCCAGACA	425	GCGACGGGUACAGGCCAGACA	1044	GGGUGUGUACCCACAGACAGCTT	1153

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GCUCAGGGCAAGAGGUCCGGC	427	GCUCAGGGCAAGAGGUCCGGC	1046	GCCAGGACUCUTUGGCCUGGGCTT	1155
CAGCUCCAGGAAGAGGUCCUG	428	CAGCUCCAGGAAGAGGUCCUG	1047	CAGGACUCUTUGGCCUGGGCTT	1156
AGCUCCAGGAAGAGGUCCUG	429	AGCUCCAGGAAGAGGUCCUG	1048	CCAGGACUCUUGCCUGGGCTT	1157
CUCCAGGAAGAGGUCCUGGU	430	CUCCAGGAAGAGGUCCUGGU	1049	AGCAGGACUCUUGCCUGGGCTT	1158
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CUGACGGUACAGGCCAGACAA	432	CUGACGGUACAGGCCAGACAA	1051	UUGUCUGGGCCUGUACCGUCAGTT	1160
CCAUUCCAUACAUUAUUGU	433	CCAUUCCAUACAUUAUUGU	1052	ACAAUAUUGGUAGGGAAUUGGTT	1161
AUUAUGGGGUACCUUGUGGA	434	AUUAUGGGGUACCUUGUGGA	1053	UCCACACGGUACCCCAUAUATT	1162
UACCCACAGACCCACCCAC	435	UACCCACAGACCCACCCAC	1054	UGGGGUUCUGGGGUUCUGGGGUATT	1163
UGUCUGGUUAUAGGUACAGC	436	UGUCUGGUUAUAGGUACAGC	1055	GUUGUUGGACUUAUACAGACATT	1164
CUUUGGAGGAGCAGGAAGCAC	437	CUUUGGAGGAGCAGGAAGCAC	1056	GUUCUUCUGGUCCUCCAAAGTT	1165
UCUUGGGAGCAGGGAGGAAGCA	438	UCUUGGGAGCAGGGAGGAAGCA	1057	UGCUUCCUGGUCCUCCAAAGATT	1166
GUUCGGGUUAUAGGUACAGCA	439	GUUCGGGUUAUAGGUACAGCA	1058	UGCGUGUUCGUACUUAUCCAGACTT	1167
GUACCCACAGACCCCAACCCA	440	GUACCCACAGACCCCAACCCA	1059	UGGGGUUCUGGGGUUCGGGUACTT	1168
UUCUUGGGAGCAGGGAGGAAGC	441	UUCUUGGGAGCAGGGAGGAAGC	1060	GUUCUCCUGGUCCUCCAAAGAATT	1169
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UGGCCUGUGGUUAUAAAUAUA	443	UGGCCUGUGGUUAUAAAUAUA	1062	UAUUTUUUAUACCAAGGCCATT	1171
UGUGCCUCUUCAGGUACCCACC	444	UGUGCCUCUUCAGGUACCCACC	1063	GGGGGUAGGCGUAAGAGGCCACATT	1172
GACGGGUACAGGCCAGACAAU	445	GACGGGUACAGGCCAGACAAU	1064	AUTUGUCUGGGCCUGUACCGUCATT	1173
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UGGGGUACUGUGGUAGAAAGA	447	UGGGGUACUGUGGUAGAAAGA	1066	UCUUUCCACACGGGUACCCCAATT	1175
GUGUACCCACAGACCCCAACC	448	GUGUACCCACAGACCCCAACC	1067	GGUGGGGGGUUCUGGGGUACACATT	1176
UAUUGGGGUACCUUGUGGGAAA	449	UAUUGGGGUACCUUGUGGGAAA	1068	UUCUCCACACGGGUACCCCAATT	1177
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GGCUCUGGGGUUAUAAAUAU	451	GGCUCUGGGGUUAUAAAUAU	1070	AUAUUTUUUAUACCAAGCCATT	1179
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CUGUGGCCUUCAGGUACCCAC	454	CUGUGGCCUUCAGGUACCCAC	1073	GUUGUAGGCGUAAGAGGCCACATT	1182
GCUGUGGGGUUAUAAAUAU	455	GCUGUGGGGUUAUAAAUAU	1074	AUAUUUUAUACCAAGCATT	1183
CCACAGACCCAAACCCACAAG	456	CCACAGACCCAAACCCACAAG	1075	CUGUGGGGUUCUGGGGUUCUGGGTT	1184
GUUCUUGGGAGCAGGGAAAG	457	GUUCUUGGGAGCAGGGAAAG	1076	CUUCUGGUUCUCCAAAGAATT	1185
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CACAGACCCAAACCCACAAGA	459	CACAGACCCAAACCCACAAGA	1078	UCUUGGGGUUCGGGUUCUGGGTT	1187

CCUGUGGCCUUCAGCUACCA	460	CCUGUGGCCUUCAGCUACCATT	1079	UGGUAGGCUAGAGGACACGGTT	1188
ACAGACCCCAACCCACAAGAA	461	ACAGACCCCAACCCACAAGAAATT	1080	UUCUUGGGGUUGGGGUUCUGUTT	1189
GGGUUUGGGAGGAGGAA	462	GGUTCUUGGGAGGAGGAAATT	1081	UUCUUGGUUCUCCAAAGAACCTT	1190
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GGGUUCUUGGGAGGAGGAA	465	GGGUUCUUGGGAGGAGGAAATT	1084	UCCUGGUGGUUCCCAAAGAACCTT	1193
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AUGAGGGACAAUUGGAGAAGU	467	AUGAGGGACAAUUGGAGAAGUTT	1086	ACUUUCUCCAAUUTGUCCCUCAUTT	1195
UGUUGCAACUCACAGUCUGG	468	UGUUGCAACUCACAGUCUGGTT	1087	CCCAAGACUGUGGUUGGCAACATT	1196
UGAGGGACAAUUGGAGAAGUG	469	UGAGGGACAAUUGGAGAAGUGTT	1088	CAUUUCUCCAAUUTGUCCCUCAUTT	1197
UGAAAUUAUUAUUAUUAAGU	470	UGAAUUUAUUAUUAUUAAGUTT	1089	ACUUUAUUAUUAUUAUUCATT	1198
GUUGCAACUCAAGUCUGGG	471	GUUGCAACUCAAGUCUGGGTT	1090	CCCCAGACUGUGGUUGGAACTT	1199
UGGAGAAGUGAAUUAUAAA	472	UGGAGAAGUGAAUUAUAAAATT	1091	UUUAUUAUUAUUCACUUCUCCATT	1200
UUGGGUTUCUUGGGAGCAGCAG	473	UUGGGUTUCUUGGGAGCAGCAGTT	1092	CUGCUGGUCCCAAGAACCCAAATT	1201
AAAGCCUAAGCCAUUGGUAA	474	AAAGCCUAAGCCAUUGGUAAATT	1093	UUACACAUGGGUUUGGCCUUTT	1202
UGGGGUUCUUGGGAGCAGCAG	475	UGGGGUUCUUGGGAGCAGCAGTT	1094	CCUGCUGGUCCCAAGAACCCATT	1203
GGAGAAGUGAAUUAUAAA	476	GGAGAAGUGAAUUAUAAAATT	1095	AUUAUUAUUAUUCACUUCUCCATT	1204
GAGAAGUGAAUUAUAAA	477	GAGAAGUGAAUUAUAAAATT	1096	UUUUUAUUAUUAUUCACUUCUCCATT	1205
AGGGACAAUUGGAGAAGUGAA	478	AGGGACAAUUGGAGAAGUGAAATT	1097	UUCACUUUCUCCAAUUTGUCCUUTT	1206
AAGUGAAUUAUUAUAAA	479	AAGUGAAUUAUUAUAAAATT	1098	UUUAUUAUUAUUAUUCACUUTT	1207
GAGGGACAAUUGGAGAAGUGA	480	GAGGGACAAUUGGAGAAGUGATT	1099	UACACUUCCAAUJUGUCCUCCATT	1208
AUAUGAGGGACAAUUGGAGAA	481	AUAUGAGGGACAAUUGGAGAATT	1100	UUCUCCAAUJUGUCCUCAUUTT	1209
AGAAGUGAAUUAUAAA	482	AGAAGUGAAUUAUAAAATT	1101	AUUAUUAUUAUUCACUUCUCCATT	1210
CUGUGUACCCACAGACCCAA	483	CUGUGUACCCACAGACCCAAATT	1102	UUGGGGUCUJUGGGGUACACAGTT	1211
GAAGUGAAUUAUUAUAAA	484	GAAGUGAAUUAUUAUAAAATT	1103	UUAAUUAUUAUUAUUCACUUCUCCATT	1212
UACAAUGUACACAGACCCAA	485	UACAAUGUACACAGACCCAAATT	1104	UAUUCUCCAAUJUGUACUUGUATT	1213
CAGUACAAUGUACACAGGAA	486	CAGUACAAUGUACACAGGAAATT	1105	UUCCAAUGUACAUJUGUACUUGUATT	1214
AAGCCUAAGCCAUGGUAAA	487	AAGCCUAAGCCAUGGUAAAATT	1106	UUUACACAUJGGCUUJAGGCCUUTT	1215
CCUUGGGGUUCUUGGGAGCAG	488	CCUUGGGGUUCUUGGGAGCAGTT	1107	GGUGGUCCCAAGAACCCAAAGGT	1216
AGUACAAUGUACACAGGAAU	489	AGUACAAUGUACACAGGAAUTT	1108	AUCCAAUGUACAUJUGUACUUTT	1217
UCAAUAAACCCUGACGGGUACAG	490	UCAAUAAACCCUGACGGGUACAGTT	1109	CUGUACCGGUACGGGUAUJUGATT	1218
ACAUUGGGAAAUAUACAUUG	491	ACAUUGGGAAAUAUACAUUGTT	1110	CCAUUGUUUUUUCCACAUUGUTT	1219
UCUUGGGGUUCUUGGGAGCAG	492	UCUUGGGGUUCUUGGGAGCAGTT	1111	CUGGUCCCAAGAACCCAAAGGATT	1220
ACGGUACAGGCCAGACAAUUA	493	ACGGUACAGGCCAGACAAUUTT	1112	UAUUGUUCGGGUACCGUUTT	1221

GUACAAUGUACACAUGGAAU	494	GUACAAUGUACACAUGGAAU	1113	AAUUCCAUGGUACATGUAC	TT	1222
UUGUCUGGUAAUGGUACAG	495	UUGUCUGGUAAUGGUACAG	1114	CUGUUGGCACUAUACAGACA	TT	1223
UAUUCAGUUUAGGGAUCAA	496	UAUUCAGUUUAGGGAUCAA	TT	1115	UUUGAUCCCAUAACUGAUATT	1224
AGUGAAUUUAUUAUAAA	497	AGUGAAUUUAUUAUAAA	TT	1116	UUUAUAAUUAUUAUUCACU	TT
GGGACAAUUGGAGAAGUGAA	498	GGGACAAUUGGAGAAGUGAA	TT	1117	AUUCACUUCUCCAAUUGGCC	TT
UUUAGGGGUACCUUGGUGGAA	499	UUUAGGGGUACCUUGGUGGAA	TT	1118	UUCCACACAGGUACCCUAU	ATT
AAGCAAUGUUAUGCCCCUCCCA	500	AAGCAAUGUUAUGCCCCUCCCA	TT	1119	UGGGAGGGCAUACAUUGC	TT
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CAAUAAACGGCUGACGGUACAGG	502	CAAUAAACGGCUGACGGUACAGG	TT	1121	CCUGUACCCGUCAGCGUUAU	GGTT
GUGCCUCUUCAGCUACCCACCG	503	GUGCCUCUUCAGCUACCCACCG	TT	1122	CGGUUGGUAGGUAGAGGGCA	CTT
GAUAAUAAUCAGUUUAGGGAU	504	GAUAAUAAUCAGUUUAGGGAU	TT	1123	AUCCCAUAUAAACUGAUUAU	ACU
						1232

**Table XII: HIV gp41 peptide sequences**

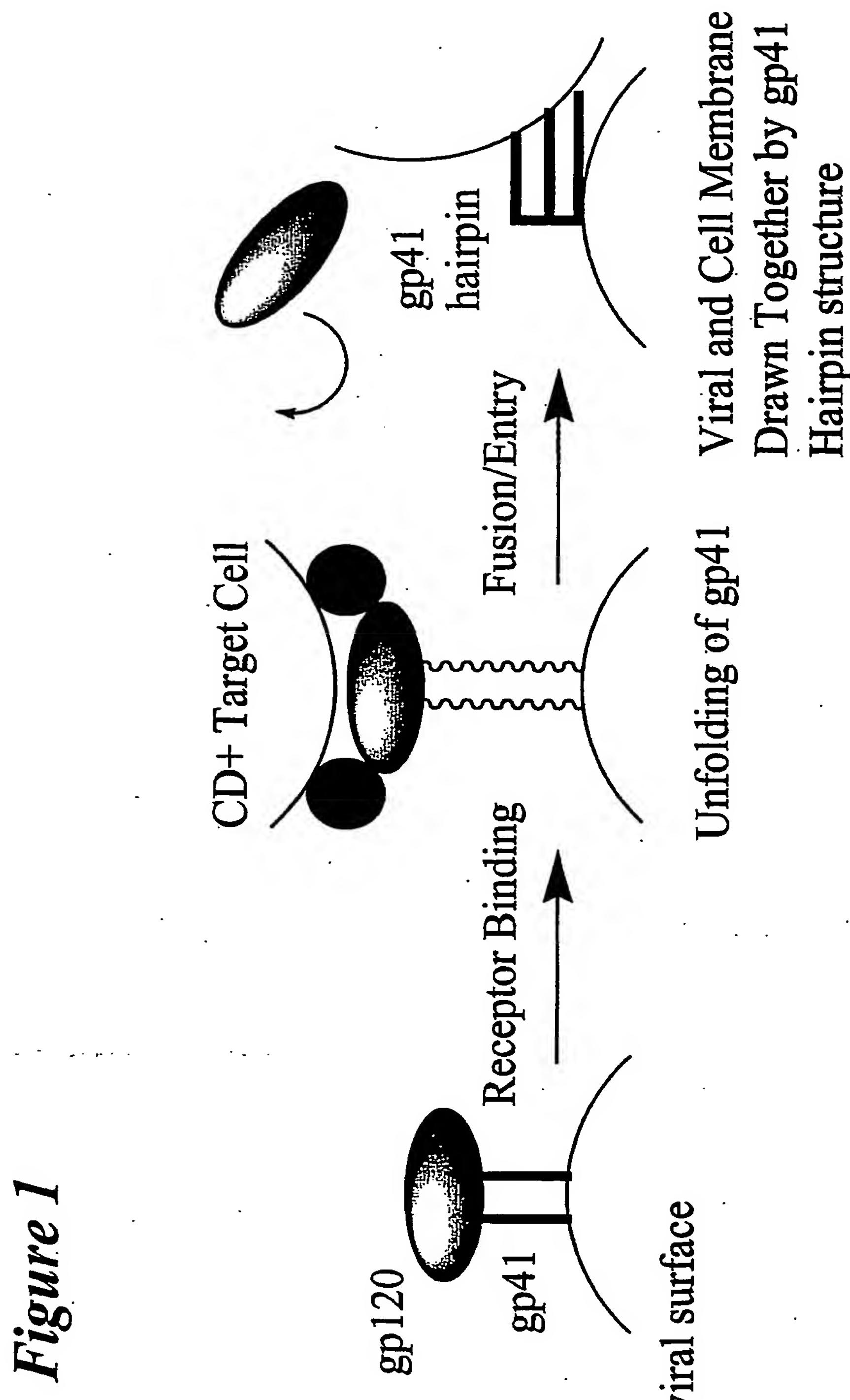
Peptide Sequence	SEQ ID NO:
YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF	1233
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CLAIMS

What we claim is:

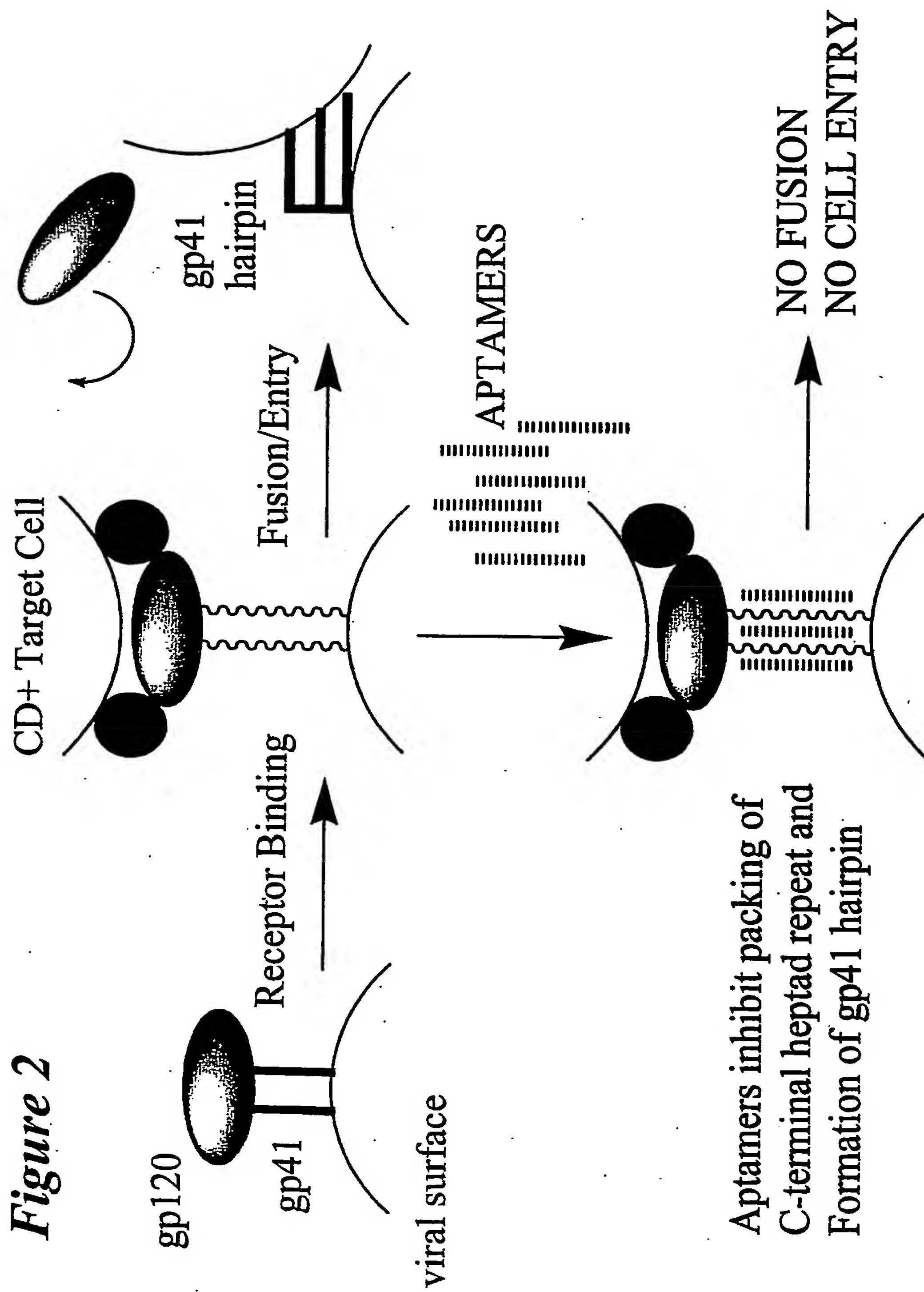
1. A short interfering RNA (siRNA) molecule that down-regulates expression of a HIV envelope glycoprotein (env) gene by RNA interference.
2. The siRNA molecule of claim 1, wherein said HIV envelope glycoprotein gene encodes sequence comprising Genbank Accession number NC\_001802.
3. The siRNA molecule of claim 1, wherein the siRNA molecule comprises sequence complementary to a nucleic acid sequence encoding HIV envelope glycoprotein or a portion thereof.
4. The siRNA molecule of claim 1, wherein said siRNA molecule comprises about 21 nucleotides.
5. The siRNA molecule of claim 1, wherein said siRNA molecule is double stranded.
6. The siRNA molecule of claim 5, wherein each strand of said siRNA molecule comprises about 21 nucleotides.
7. The siRNA molecule of claim 1, wherein said siRNA molecule has anti-fusogenic activity against HIV entry into a cell.
8. The siRNA molecule of claim 1, wherein said siRNA molecule is chemically synthesized.
9. The siRNA molecule of claim 1, wherein said siRNA molecule comprises at least one nucleic acid sugar modification.
10. The siRNA molecule of claim 1, wherein said siRNA molecule comprises at least one nucleic acid base modification.
11. The siRNA molecule of claim 1, wherein said siRNA molecule comprises at least one nucleic acid backbone modification.
12. A method for modulating HIV cell fusion activity in a cell comprising administering to said cell the siRNA molecule of claim 1 under conditions suitable for modulating said HIV cell fusion activity.

13. The method of claim 12, wherein said cell is a mammalian cell.
14. The method of claim 13, wherein said mammalian cell is a human cell.
15. A method of treating HIV-1 infection in a subject comprising administering to the subject the siRNA of claim 1 under conditions suitable for said treatment.
16. The method of claim 15, wherein said administration is in the presence of a delivery reagent.
17. The method of claim 16, wherein said delivery reagent is a lipid.
18. The method of claim 17, wherein said lipid is a cationic lipid.
19. The method of claim 16, wherein said delivery reagent is a liposome.
20. A composition comprising the siRNA of claim 1 and a pharmaceutically acceptable carrier or diluent.



*Figure 1*

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